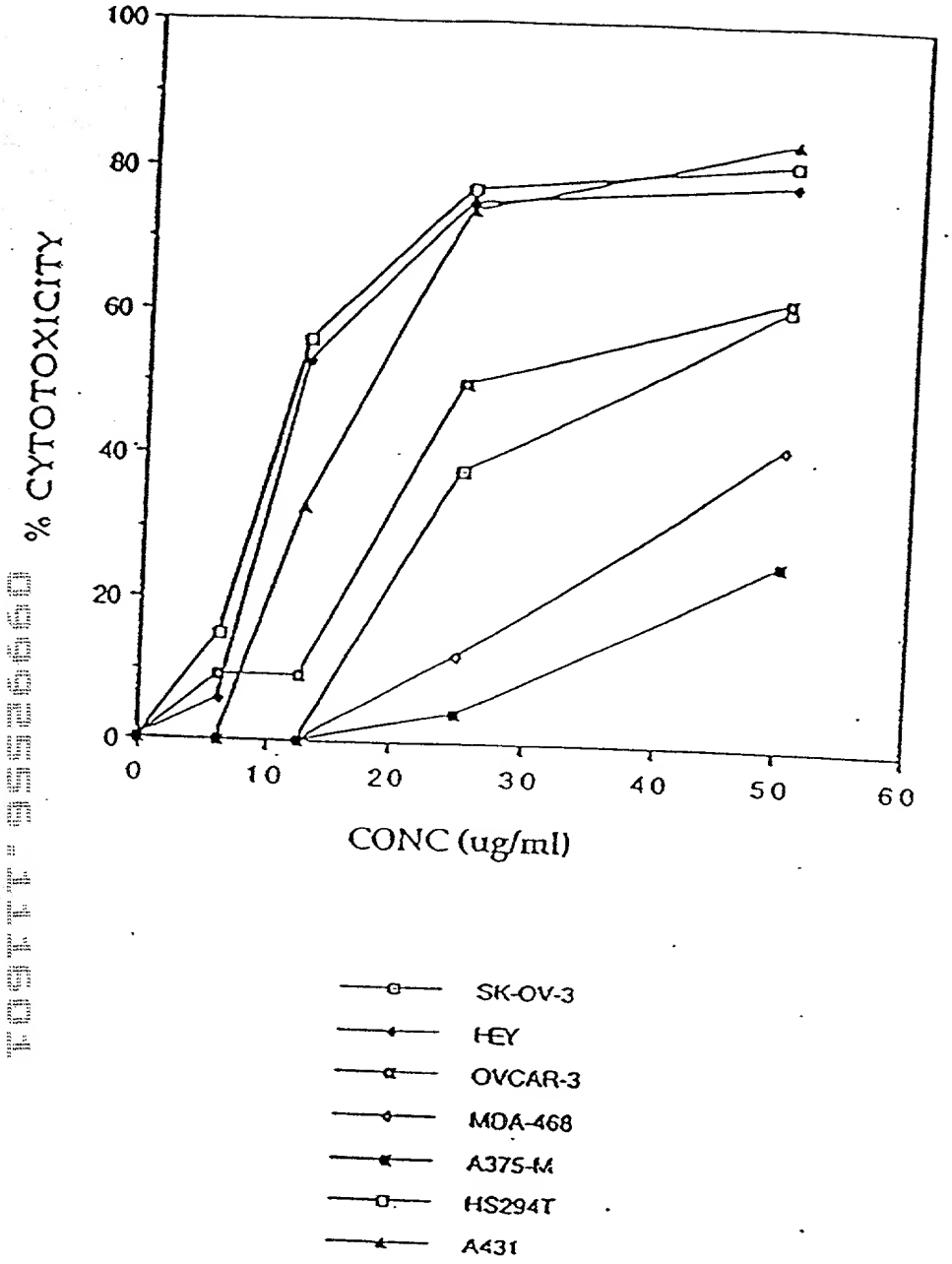

% CYTOTOXICITY
100
80
60
40
20
0
0
10
20
30
40
50
60
CONC (ug/ml)
SK-OV-3
HEY
OVCAR-3
MDA-468
A375-M
HS294T
A431

FIG. 1

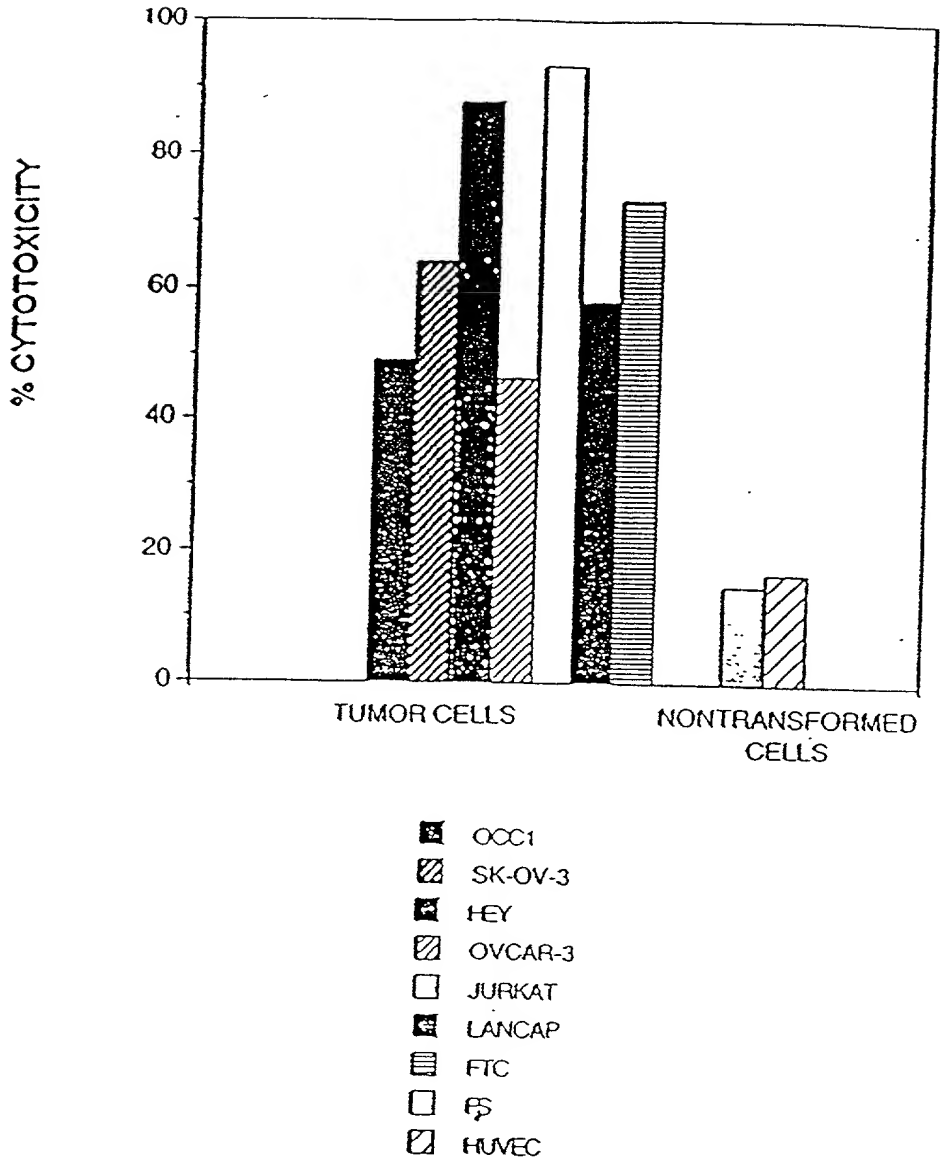

% CYTOTOXICITY
100
80
60
40
20
0
TUMOR CELLS
NONTRANSFORMED CELLS
OCC1
SK-OV-3
HEY
OVCAR-3
JURKAT
LANCAP
FTC
HUVEC

FIG. 2

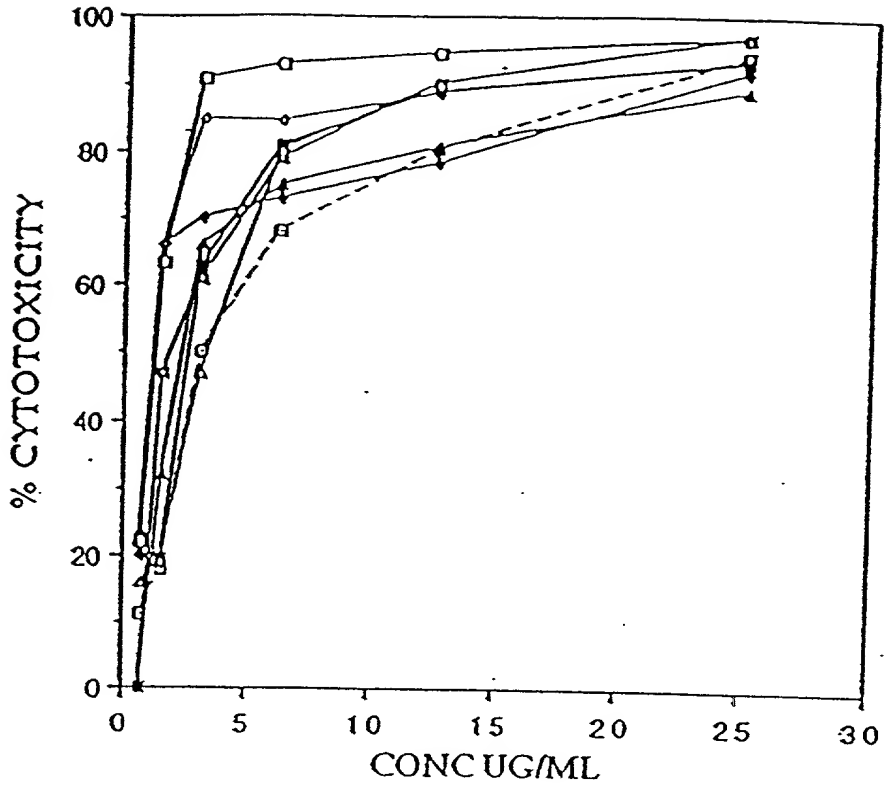

100
80
60
40
20
0
% CYTOTOXICITY
0
5
10
15
20
25
30
CONC UG/ML
HEY
OVCAR-3
C-1
SKOV-3
PANC-1
769-P
786-0
A498

FIG. 3

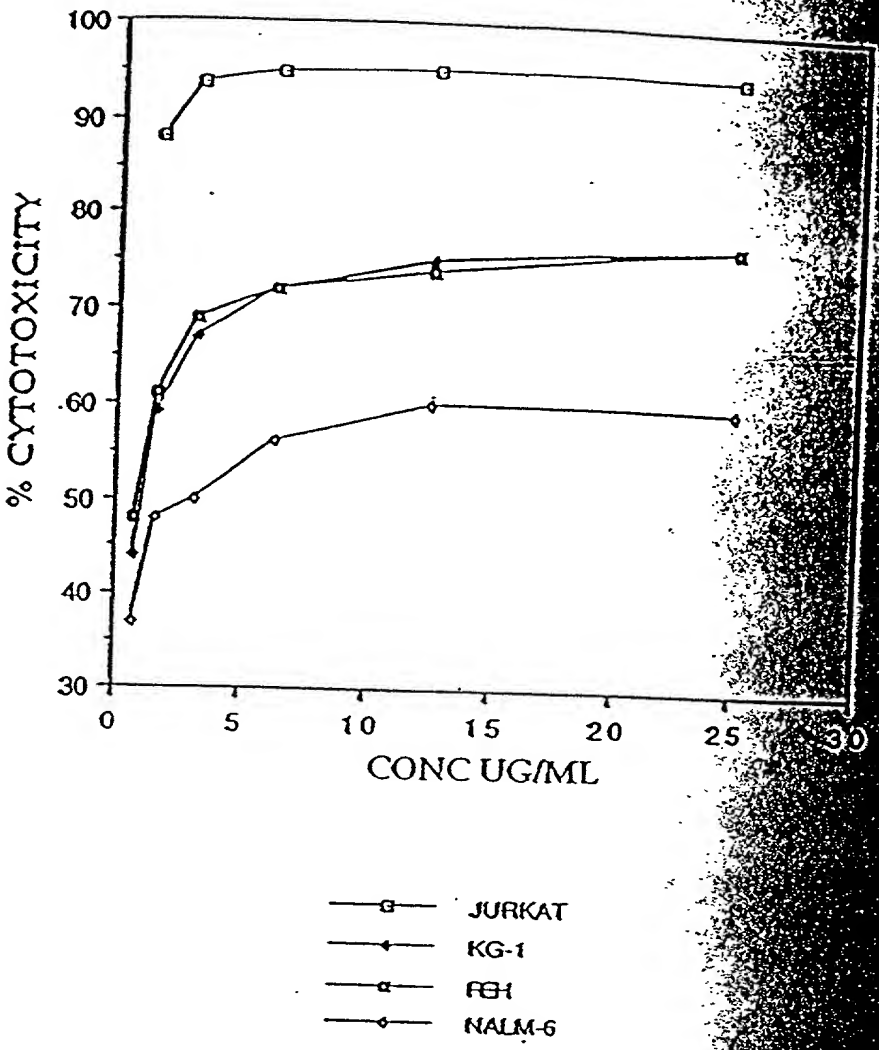

100
90
80
70
60
50
40
30
% CYTOTOXICITY
0
5
10
15
20
25
30
CONC UG/ML
JURKAT
KG-1
REH
NALM-6

FIG. 4

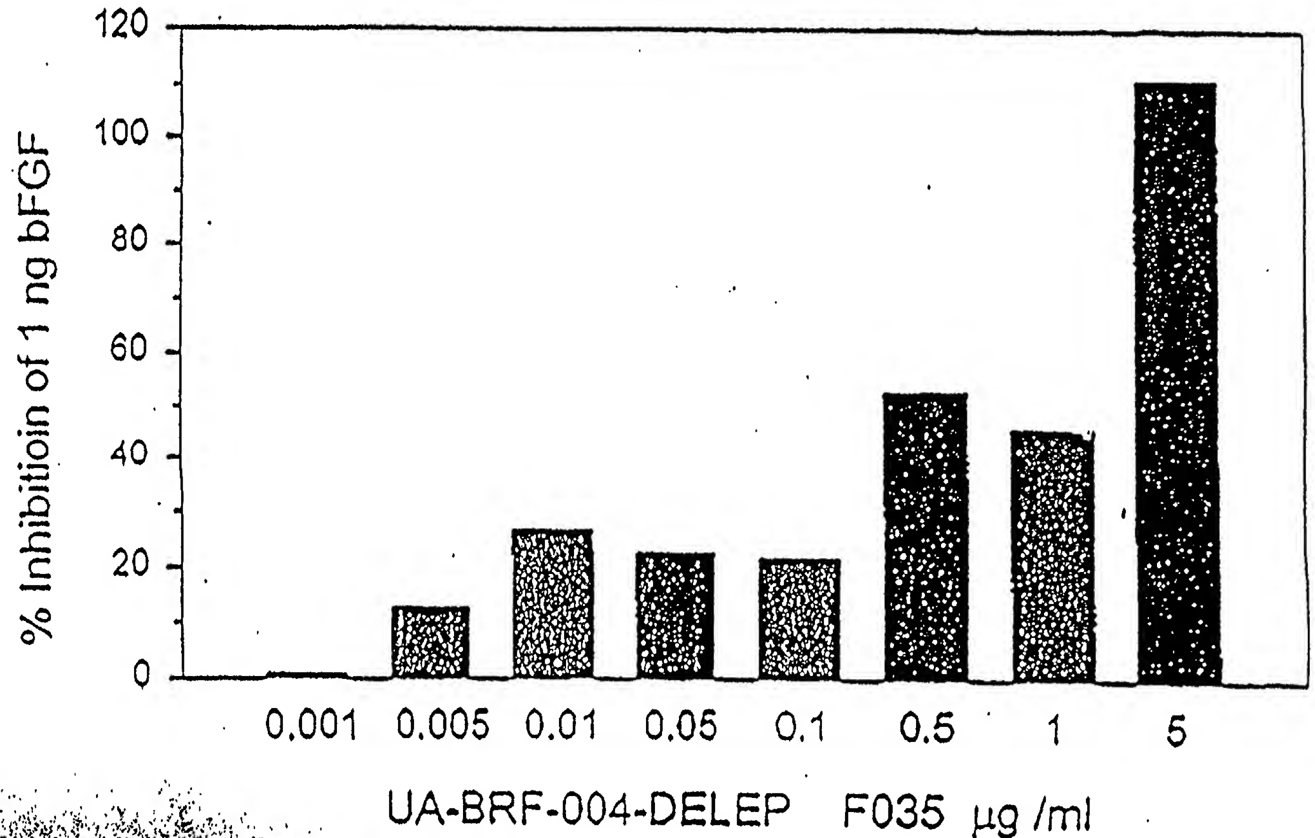

120
100
80
60
40
20
0
% Inhibitioin of 1 ng bFGF
0.001
0.005
0.01
0.05
0.1
0.5
1
5
UA-BRF-004-DELEP F035 μg /ml

FIG. 5

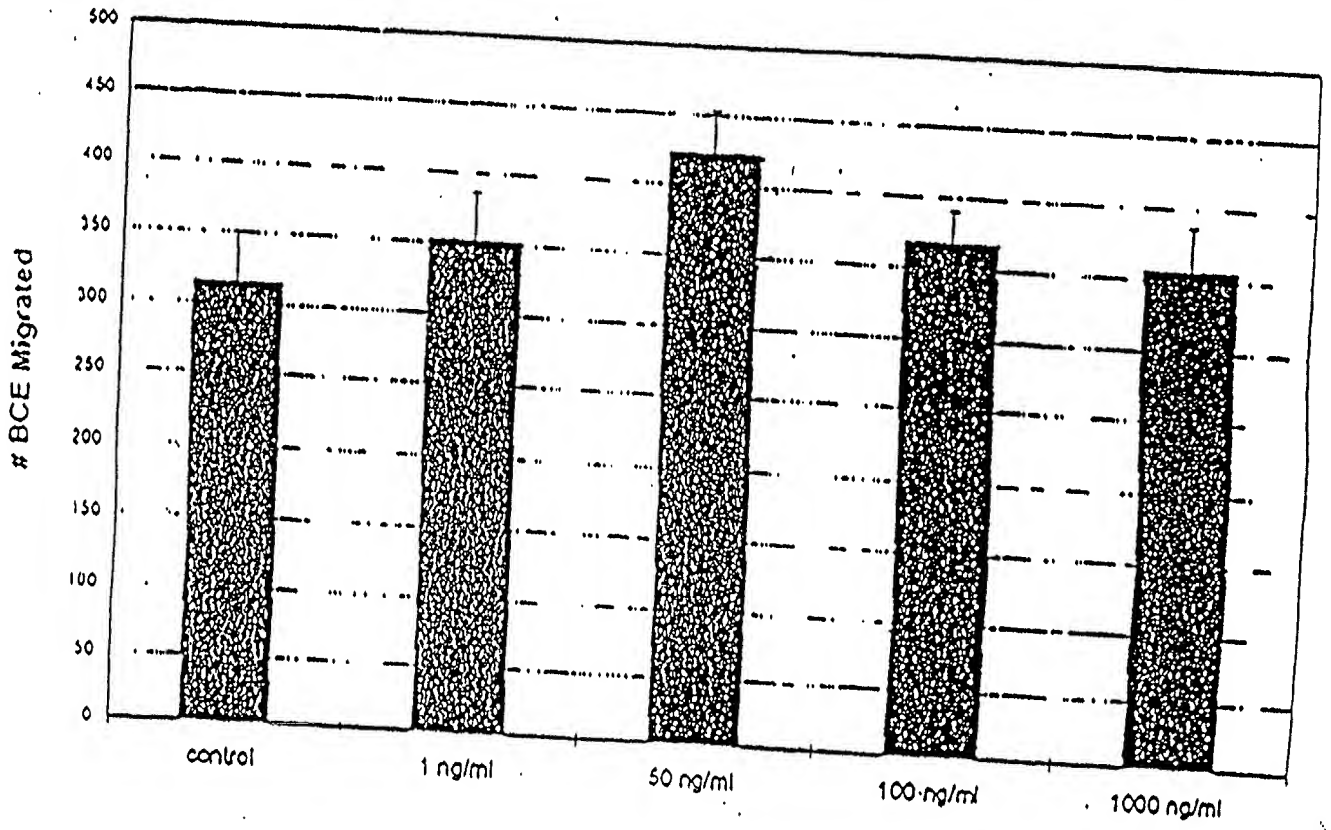

BCE Migrated
500
450
400
350
300
250
200
150
100
50
0
control
1 ng/ml
50 ng/ml
100 ng/ml
1000 ng/ml

FIG. 6

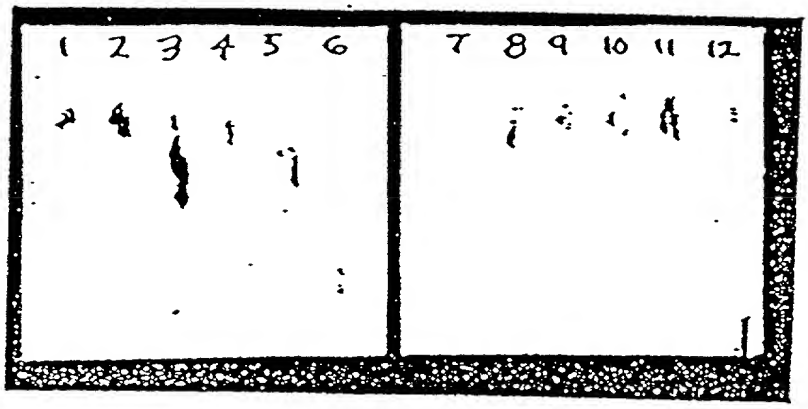


FIG. 7

FIG. 8

Dose: 0.2 ml

ACETONE
4 weeks

Mouse # 133

FIG. 9A

Dose: 0.2 ml

ACETONE
8 Weeks

Mouse # 150

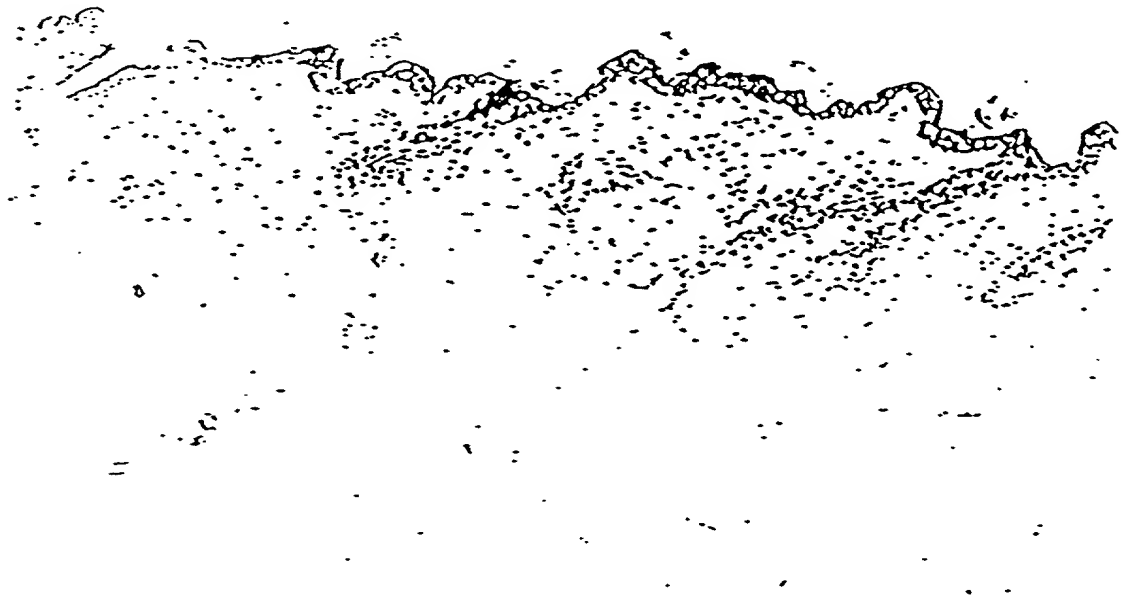


FIG. 9B

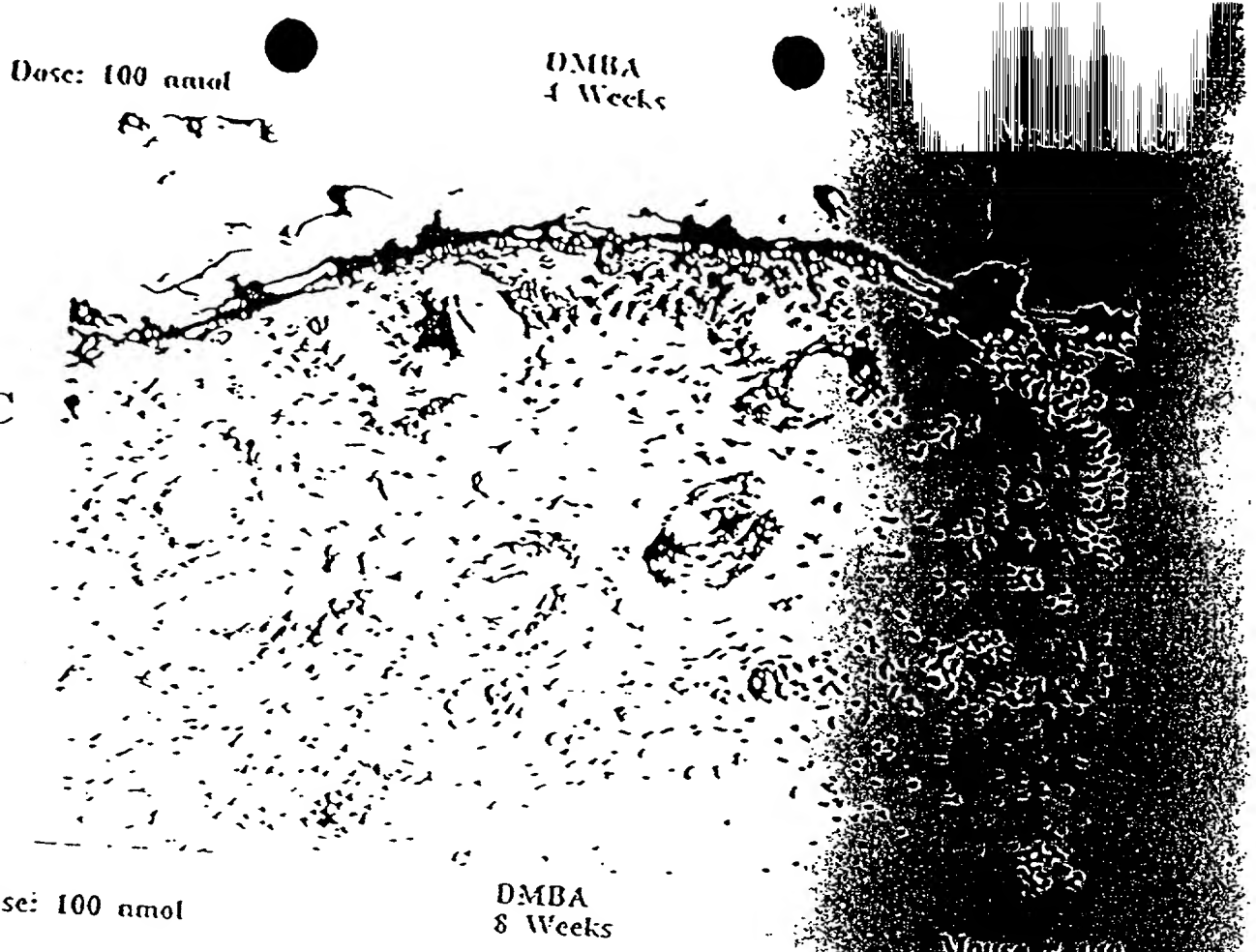

Dose: 100 nmol
DMBA
4 Weeks

FIG. 9C

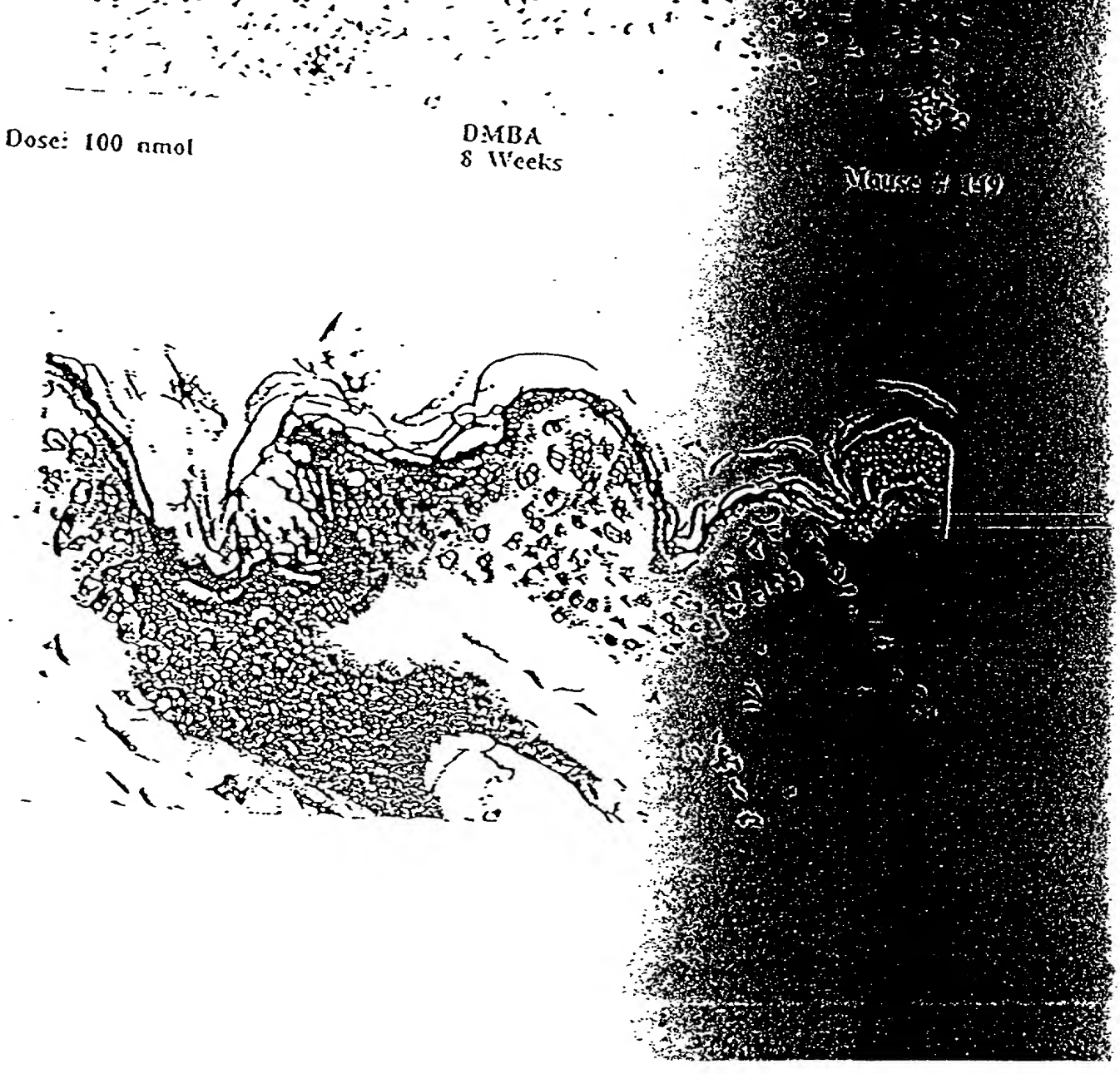

Dose: 100 nmol
DMBA
8 Weeks
Mouse # 149

FIG. 9D

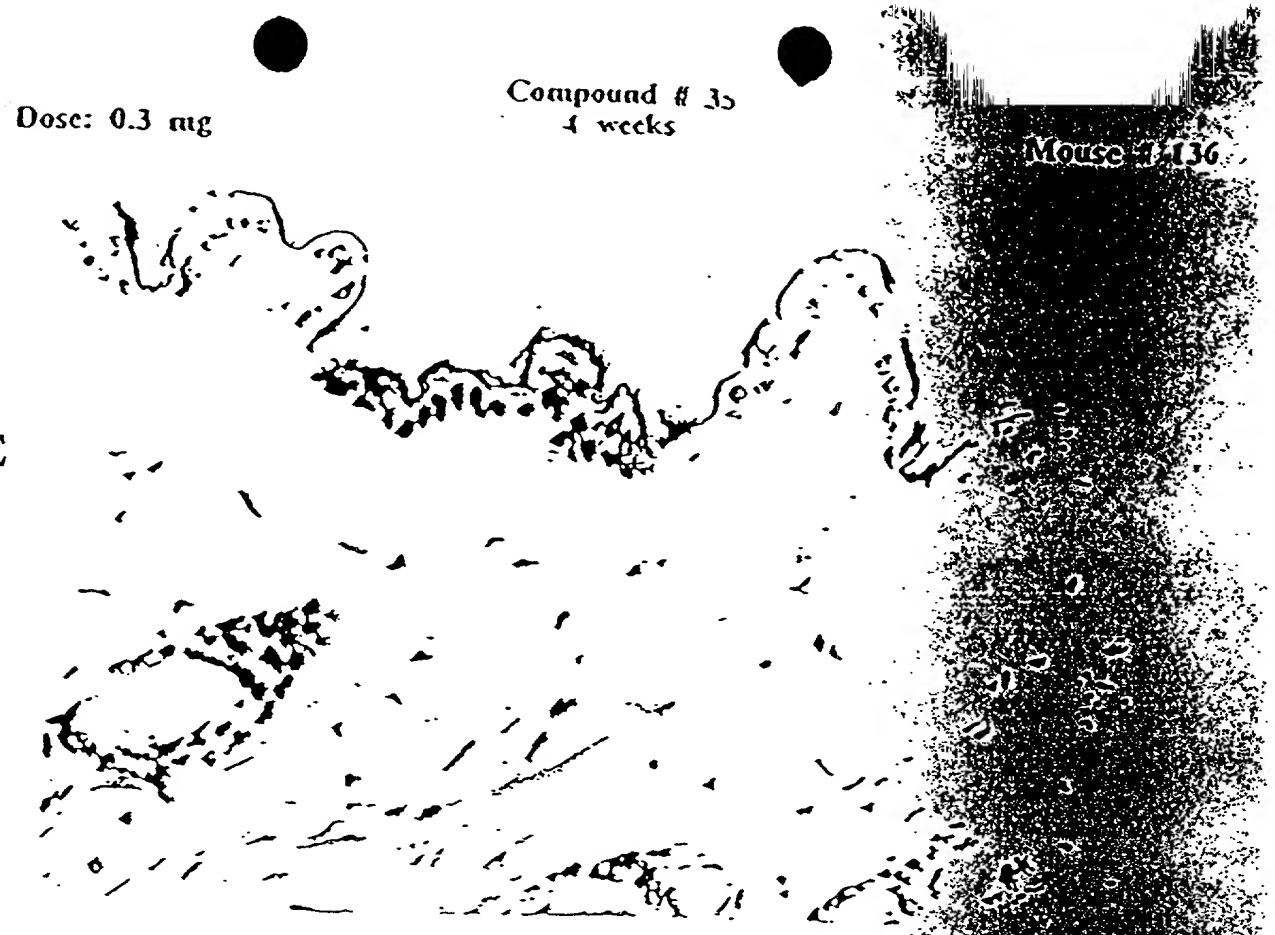

Dose: 0.3 mg
Compound # 35
4 weeks
Mouse # 136

FIG. 9E

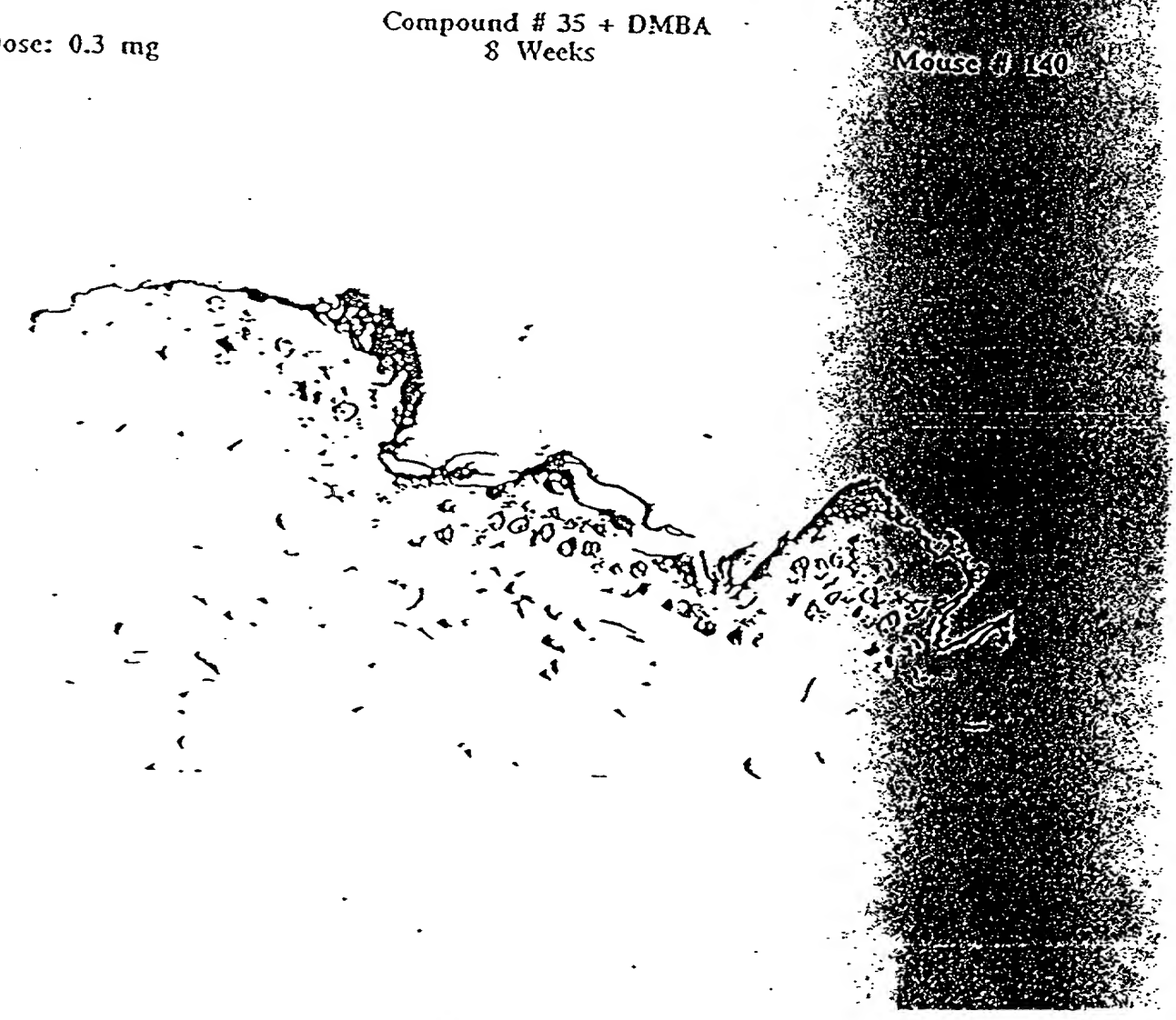
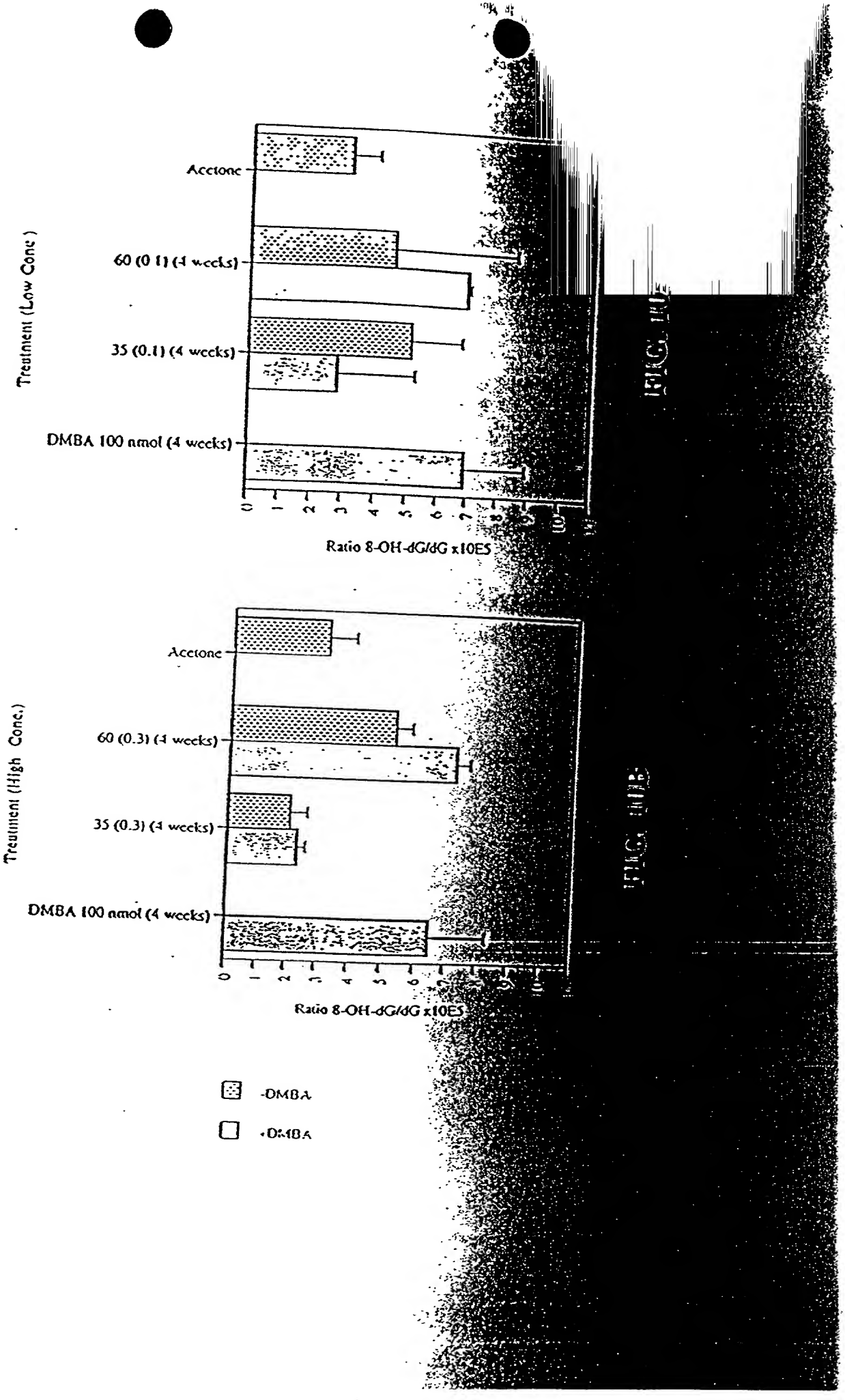

Dose: 0.3 mg
Compound # 35 + DMBA
8 Weeks
Mouse # 140

FIG. 9F


Acetone
60 (0.1) (4 weeks)
35 (0.1) (4 weeks)
DMBA 100 nmol (4 weeks)
Treatment (Low Conc.)
0 1 2 3 4 5 6 7 8 9 10
Ratio 8-OH-dG/dG x10E5
Acetone
60 (0.3) (4 weeks)
35 (0.3) (4 weeks)
DMBA 100 nmol (4 weeks)
Treatment (High Conc.)
0 1 2 3 4 5 6 7 8 9 10
Ratio 8-OH-dG/dG x10E5
-DMBA
+DMBA

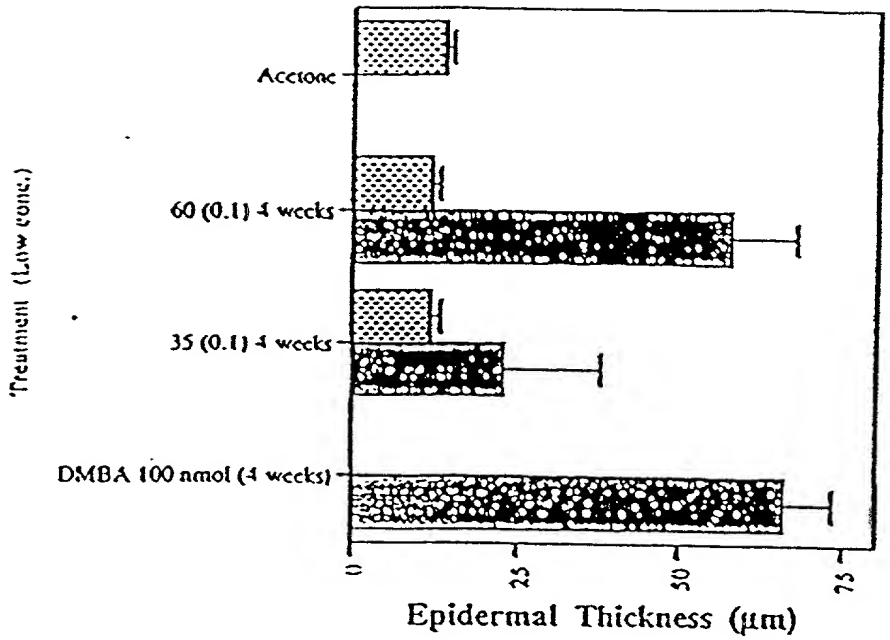


FIG. 11A

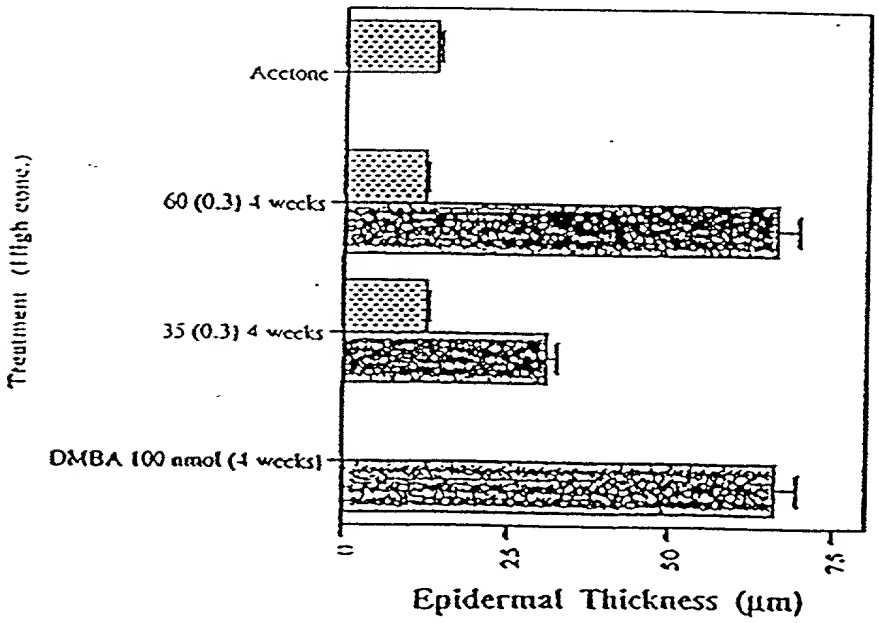
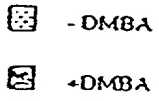


FIG. 11B



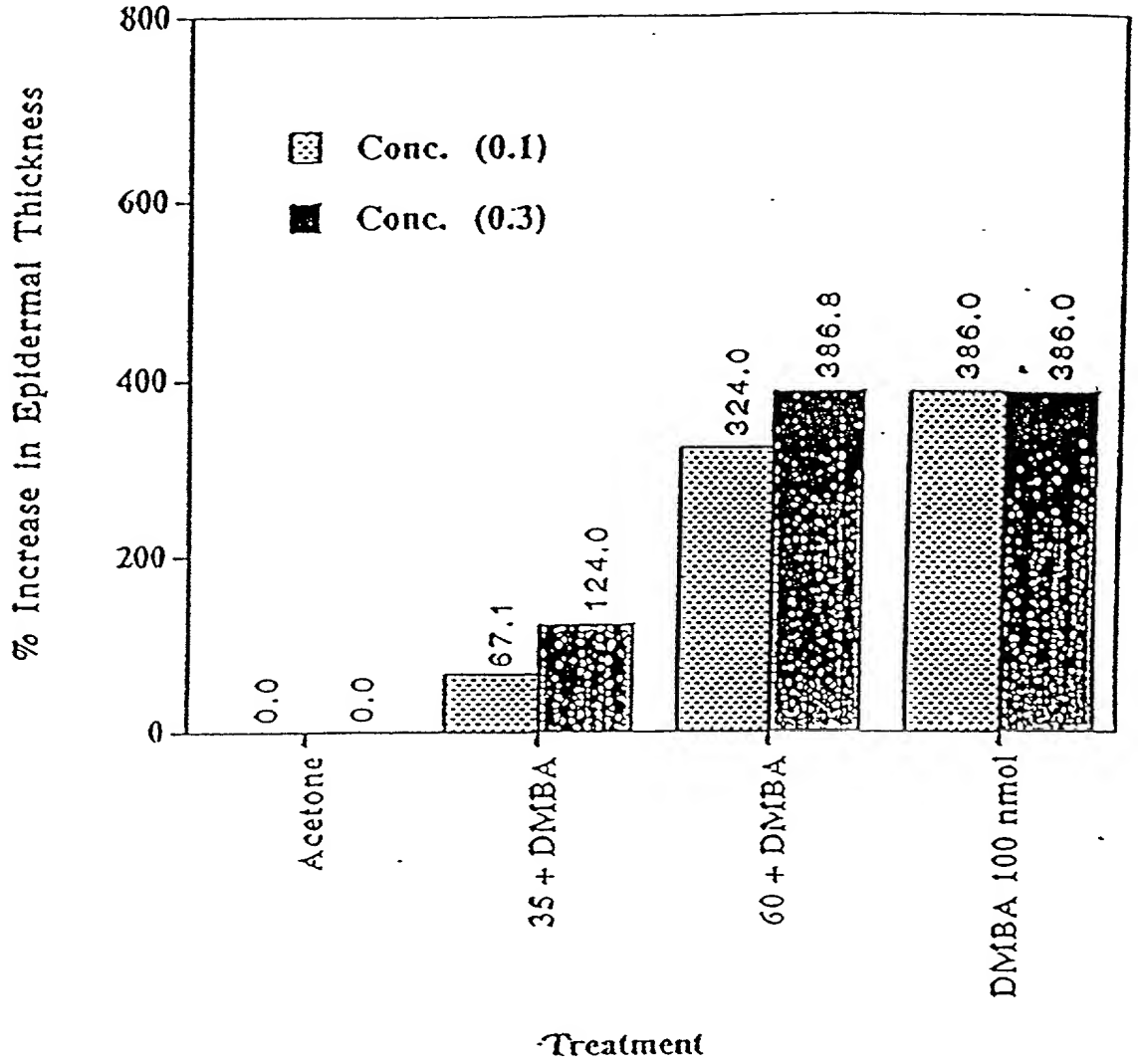

800
600
400
200
0
% Increase in Epidermal Thickness
Conc. (0.1)
Conc. (0.3)
0.0
0.0
67.1
124.0
324.0
386.8
386.0
386.0
Acetone
35 + DMBA
60 + DMBA
DMBA 100 nmol
Treatment

FIG. 12

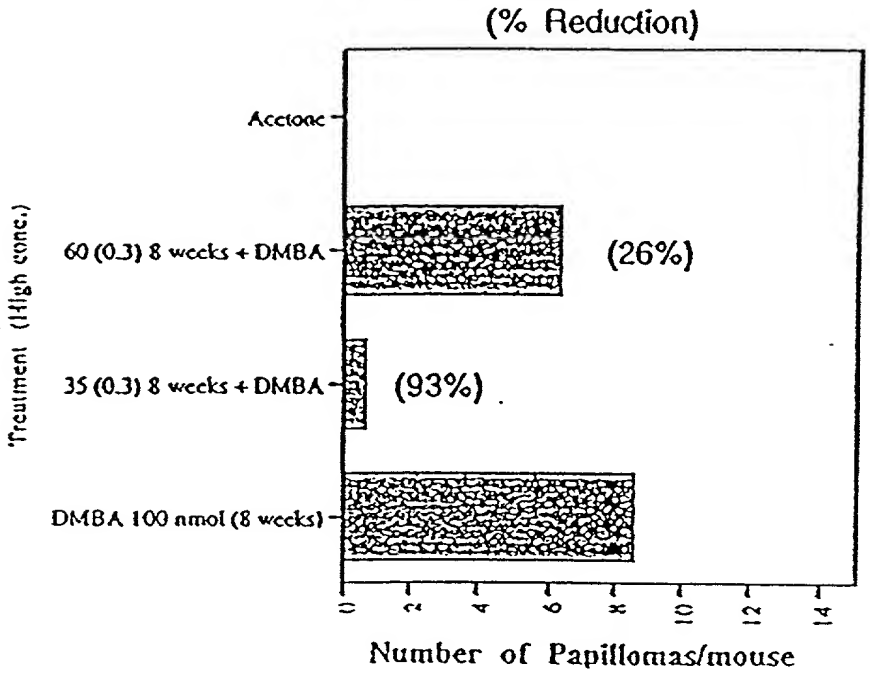

(% Reduction)
Acetone
60 (0.3) 8 weeks + DMBA
(26%)
35 (0.3) 8 weeks + DMBA
(93%)
DMBA 100 nmol (8 weeks)
Treatment (High conc.)
0
2
4
6
8
10
12
14
Number of Papillomas/mouse

FIG. 13

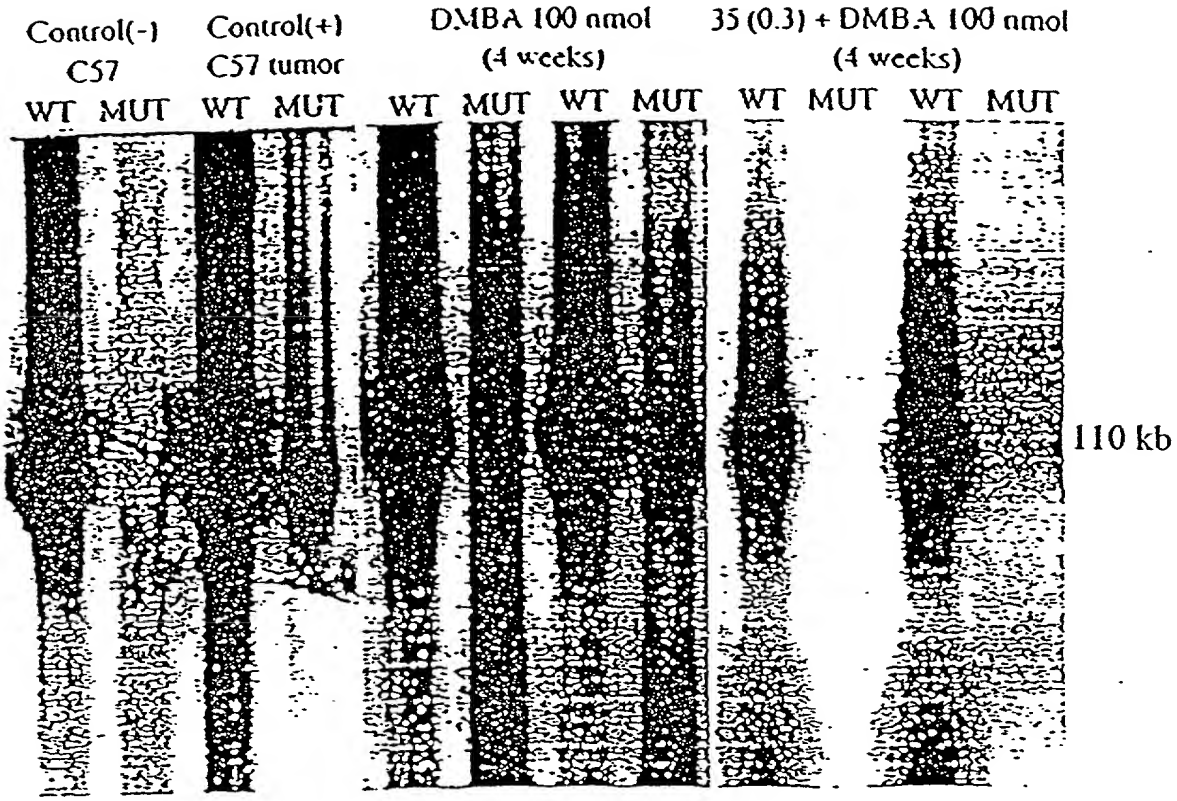

Control(-)
C57
Control(+)
C57 tumor
DMBA 100 nmol
(4 weeks)
35 (0.3) + DMBA 100 nmol
(4 weeks)
WT MUT WT MUT WT MUT WT MUT WT MUT WT MUT
110 kb

FIG. 14

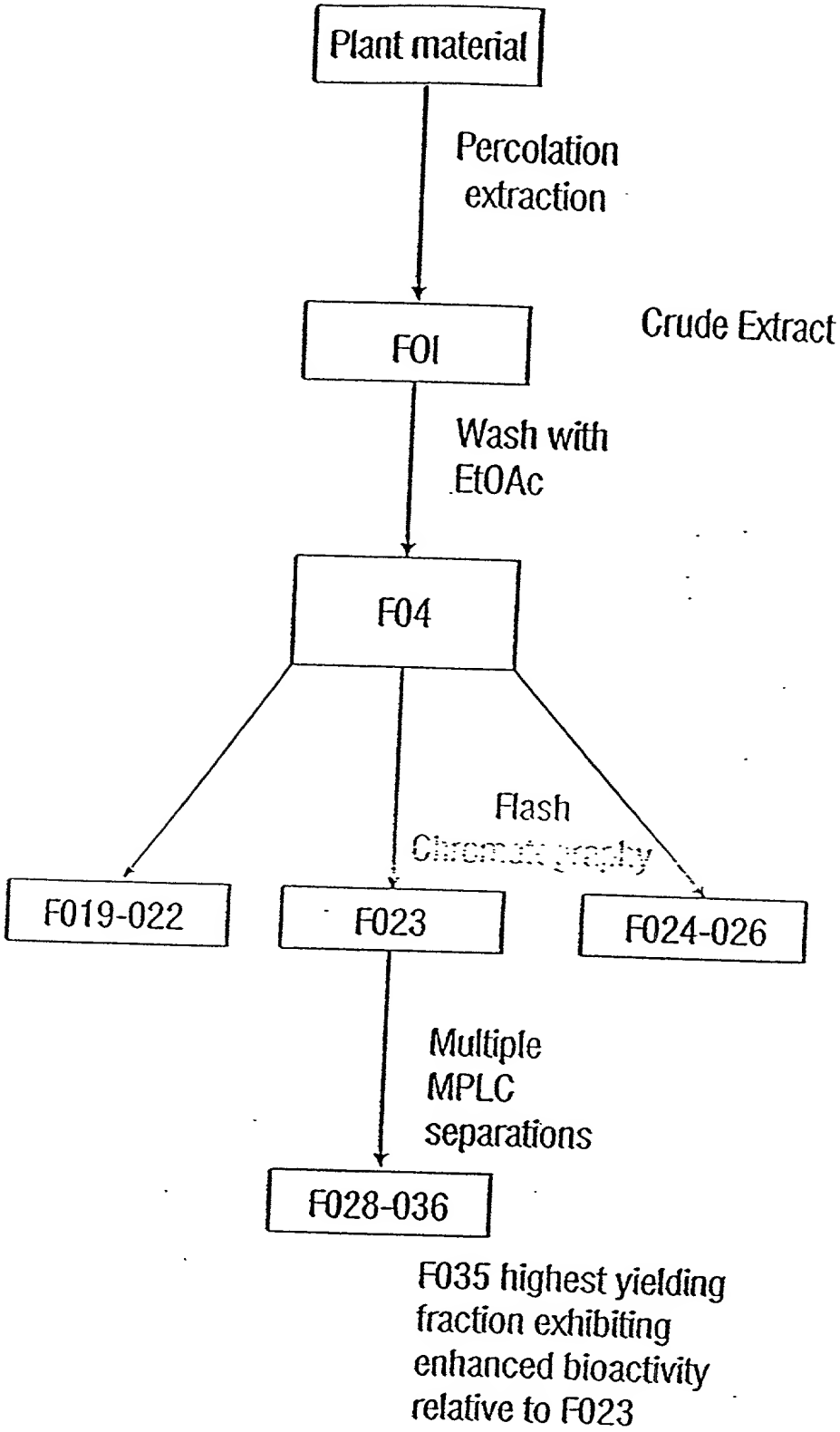

Plant material
Percolation extraction
F01
Crude Extract
Wash with EtOAc
F04
Flash
F019-022
F023
F024-026
Multiple MPLC separations
F028-036
F035 highest yielding fraction exhibiting enhanced bioactivity relative to F023

FIG. 15

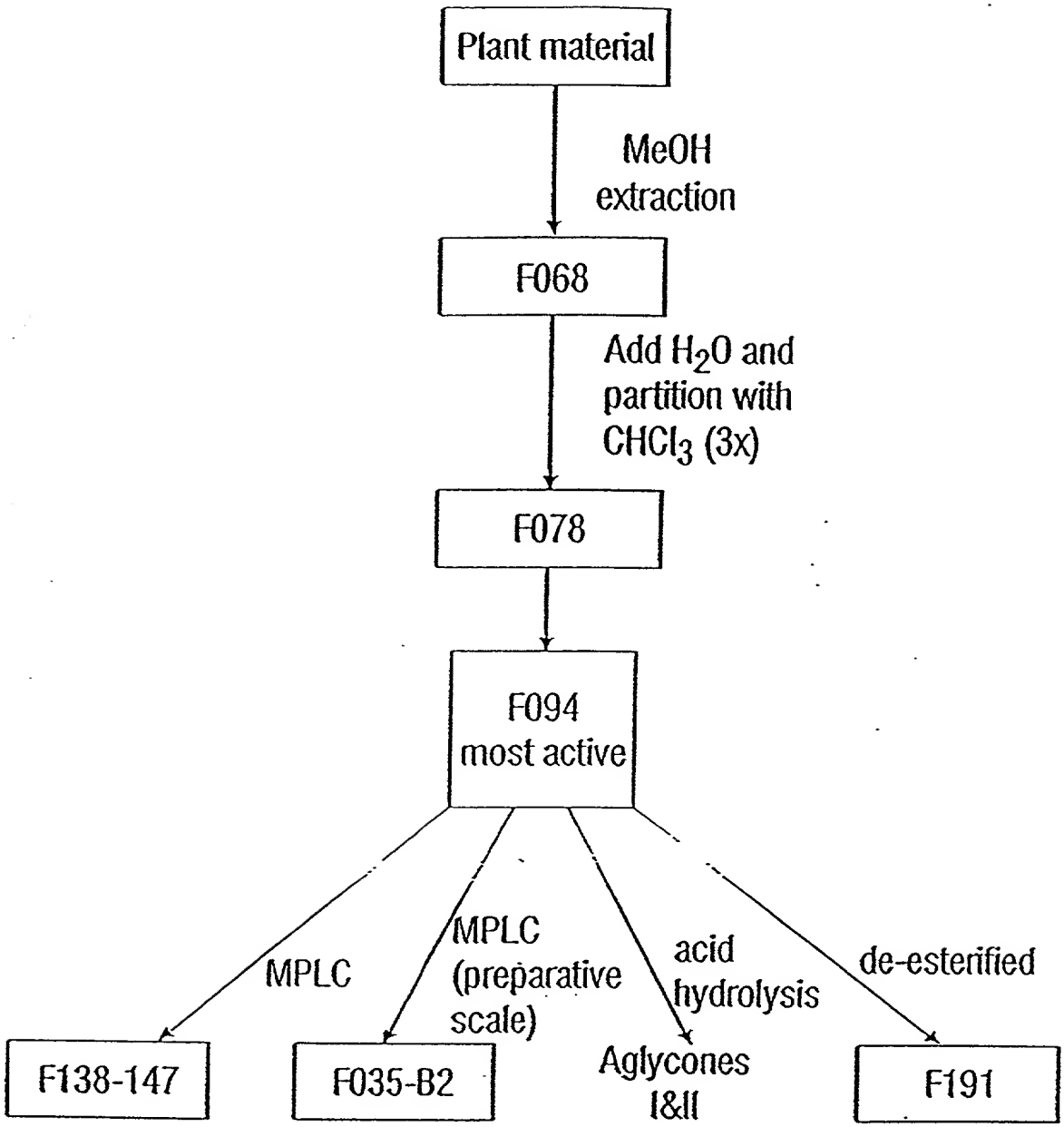

Plant material
MeOH extraction
F068
Add H_2O and partition with $CHCl_3$ (3x)
F078
F094 most active
MPLC
MPLC (preparative scale)
acid hydrolysis
de-esterified
F138-147
F035-B2
Aglycones I&II
F191

FIG. 16

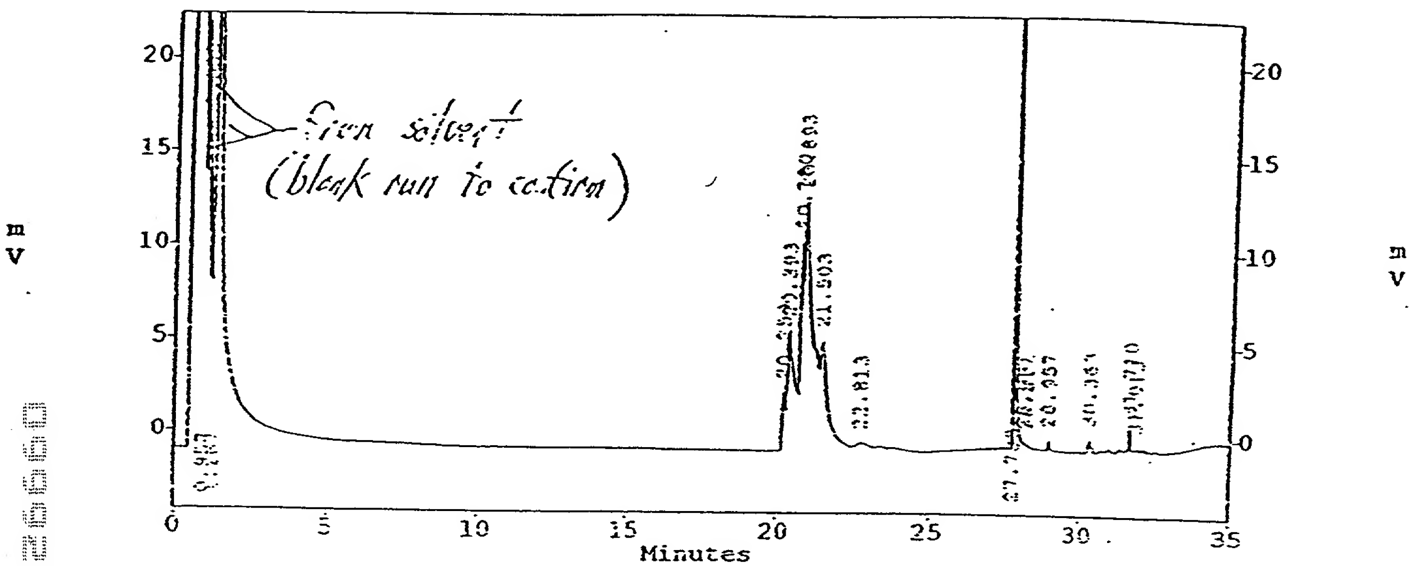

mV
20
15
10
5
0
from solvent
(blank run to confirm)
0
5
10
15
20
25
30
35
Minutes
20
15
10
5
0
mV

FIG. 17A

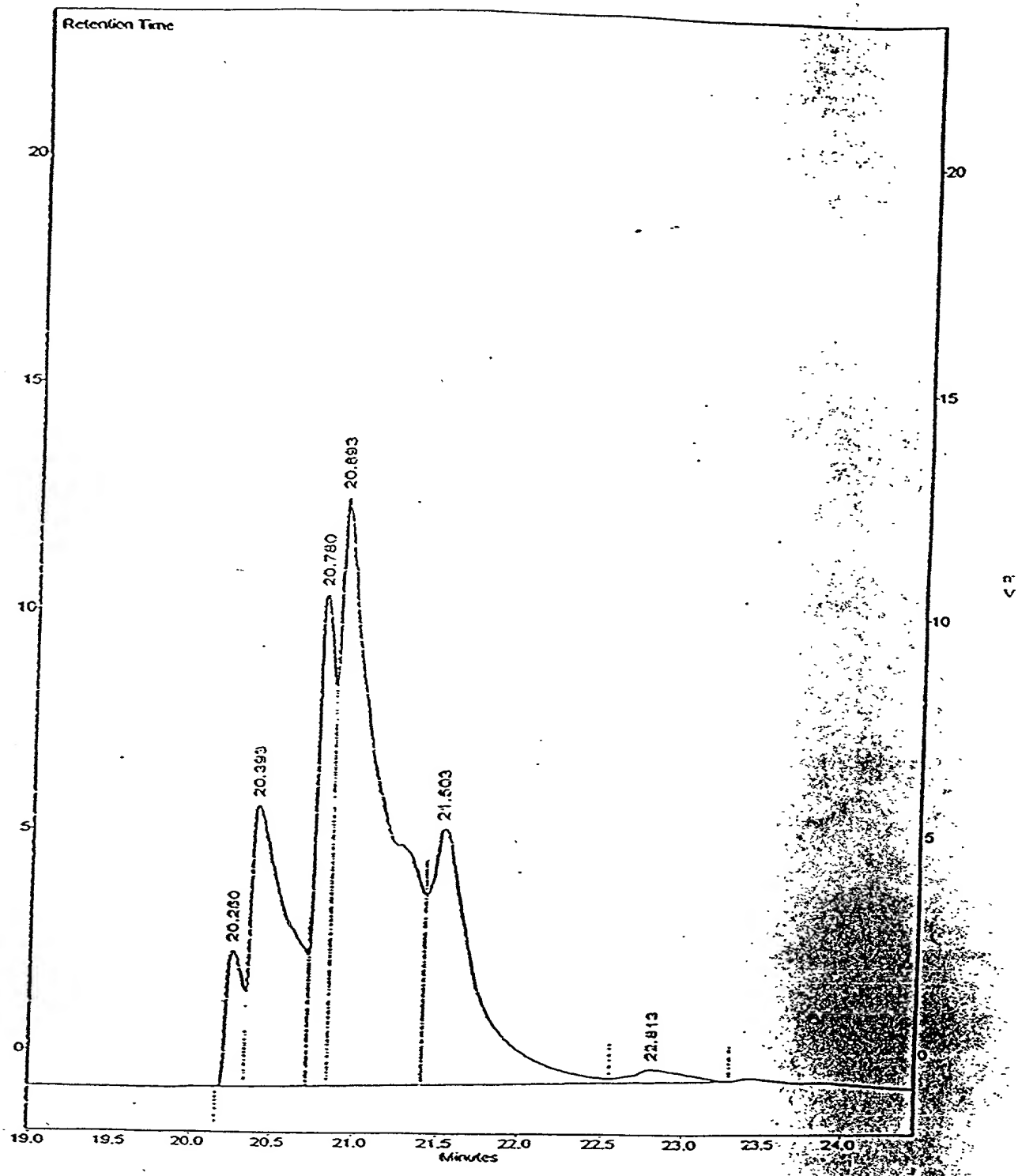

Retention Time
20.260
20.393
20.780
20.893
21.503
22.813
Minutes
19.0
19.5
20.0
20.5
21.0
21.5
22.0
22.5
23.0
23.5
24.0
0
5
10
15
20

FIG. 17B

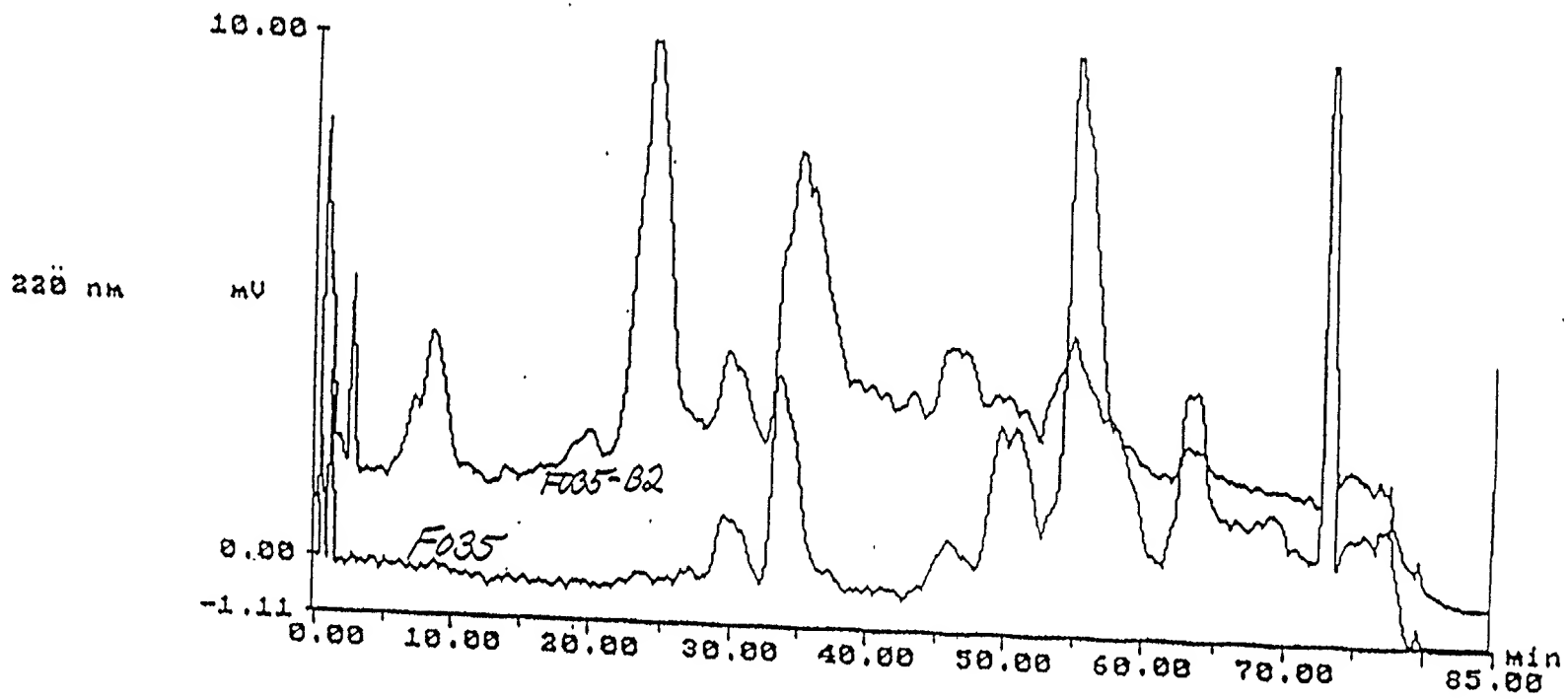

10.00
220 nm
mV
F035-B2
F035
0.00
-1.11
0.00
10.00
20.00
30.00
40.00
50.00
60.00
70.00
85.00
min

FIG. 18A

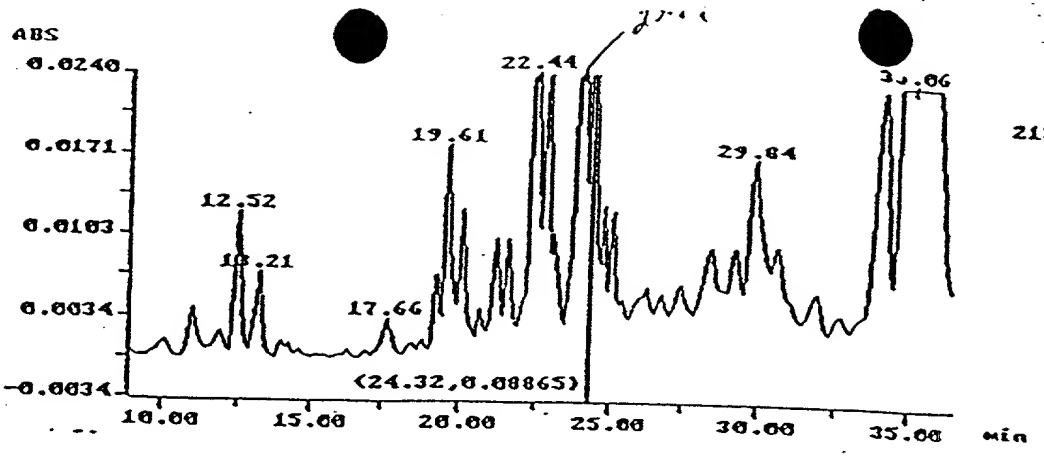


FIG. 18B

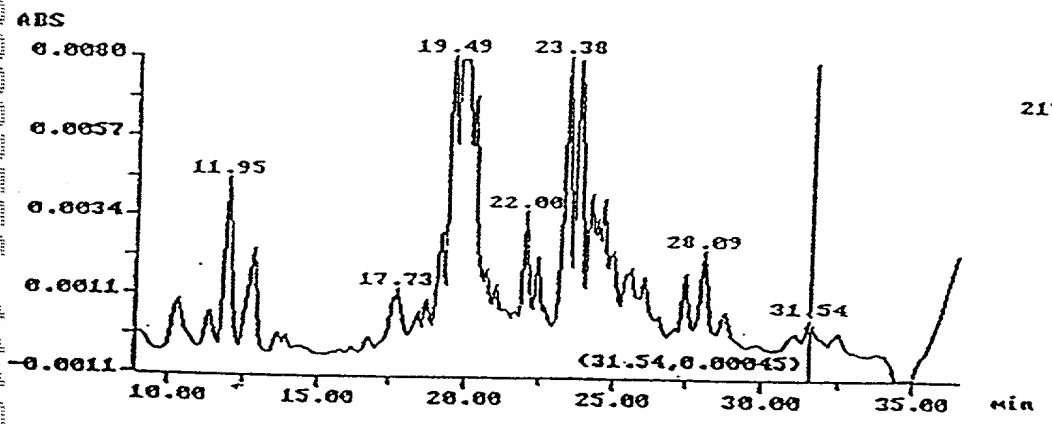


FIG. 18C

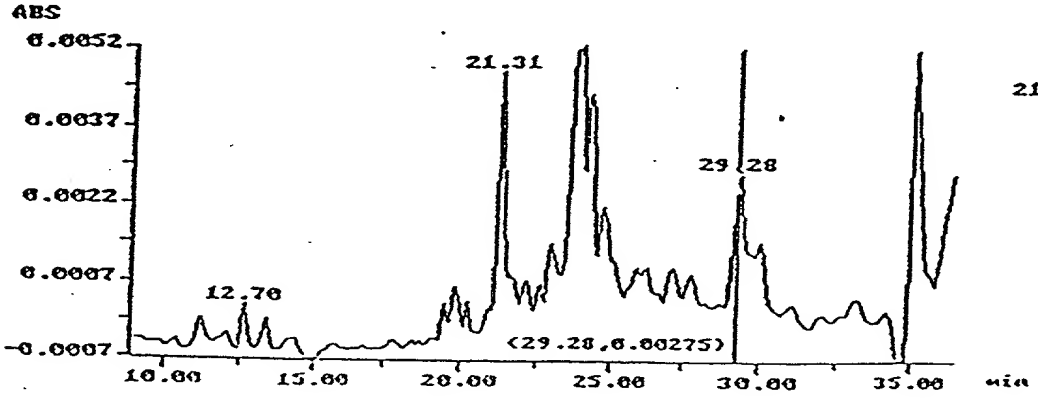


FIG. 18D

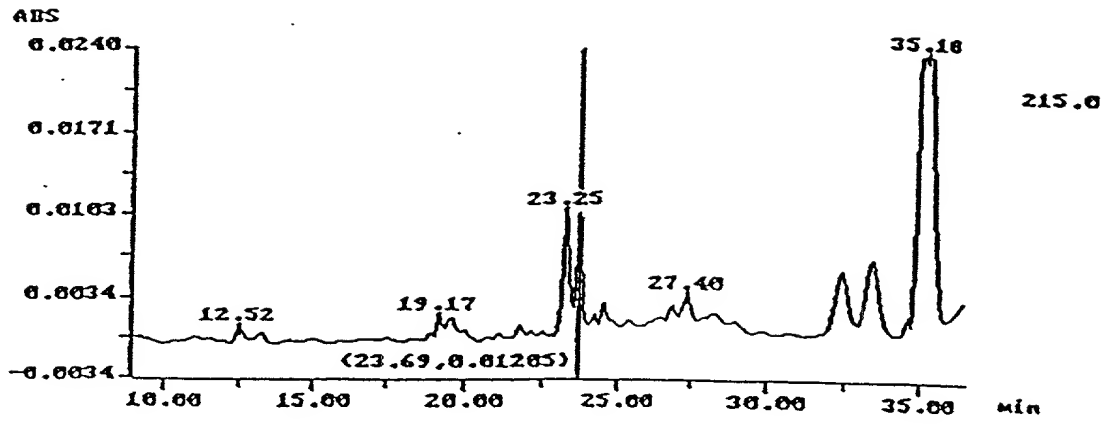


FIG. 18E

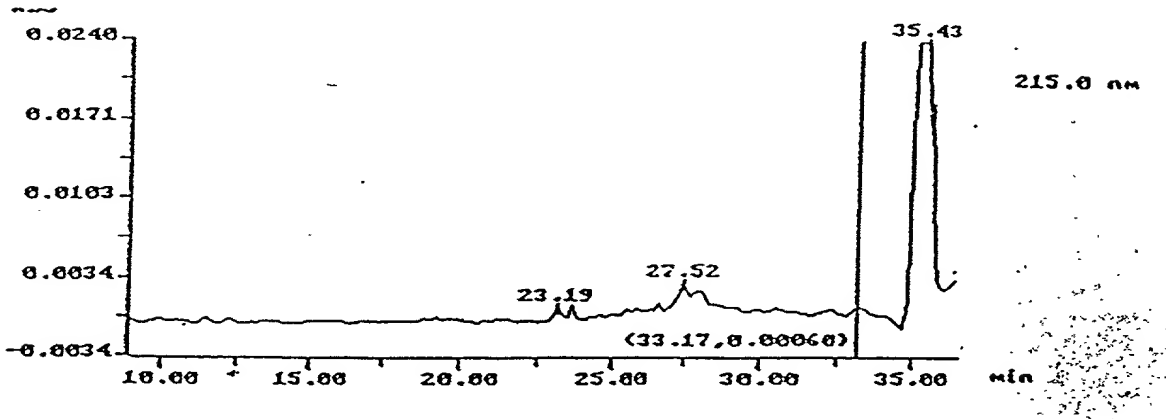


FIG. 18F

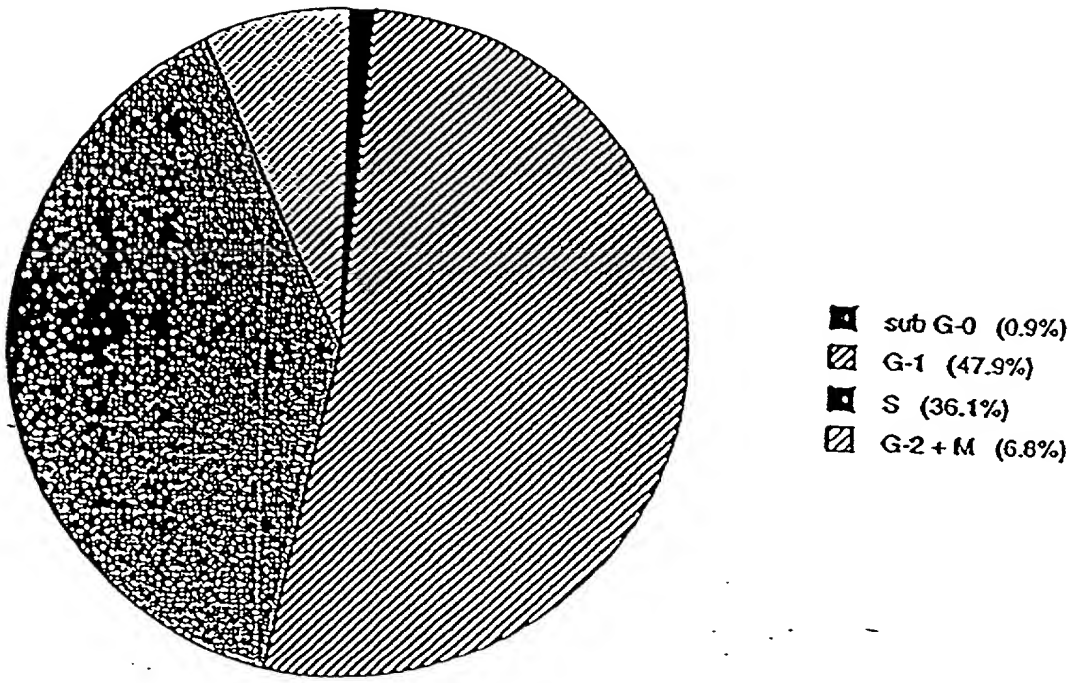


FIG. 19A

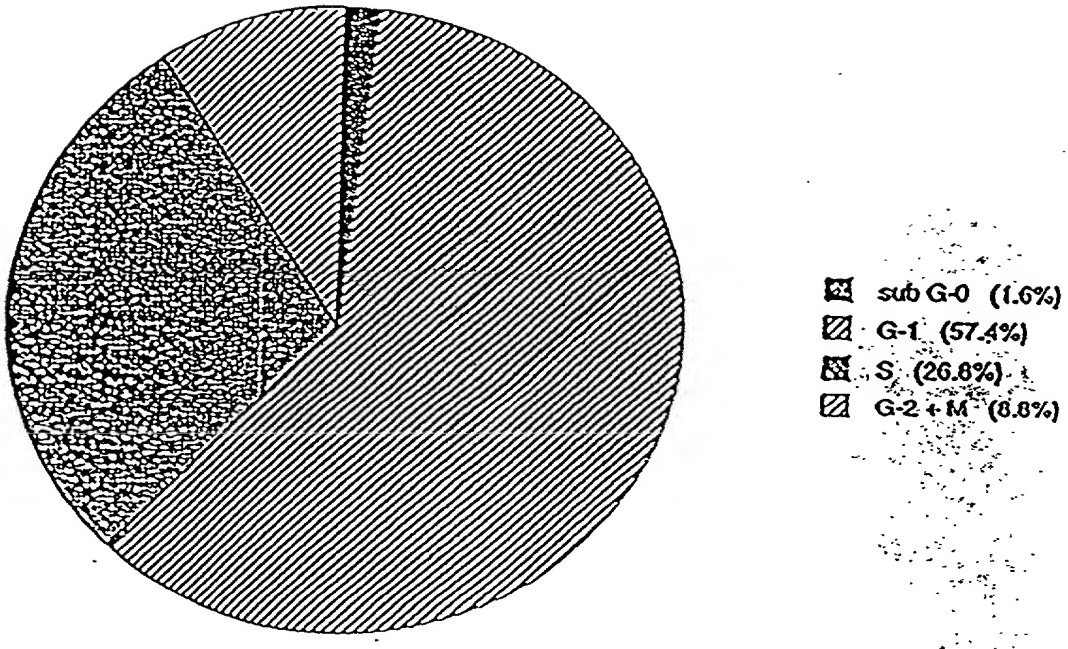
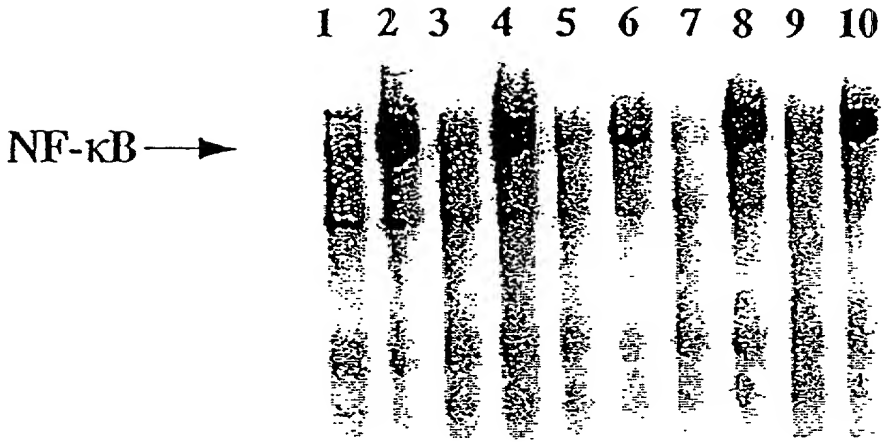


FIG. 19B



1 :Untreated
2 :TNF (100pM)
3 : F035 (1ug/ml)
4 : TNF+ F035 (1ug/ml)
5 : F035 (2μg/ml)
6 : TNF+ F035 (2μg/ml)
7 : F094 (1μg/ml)
8 : TNF+F094 (1μg/ml)
9 : F094 (2ug/ml)
10 : TNF+ F094 (2ug/ml)

FIG. 20

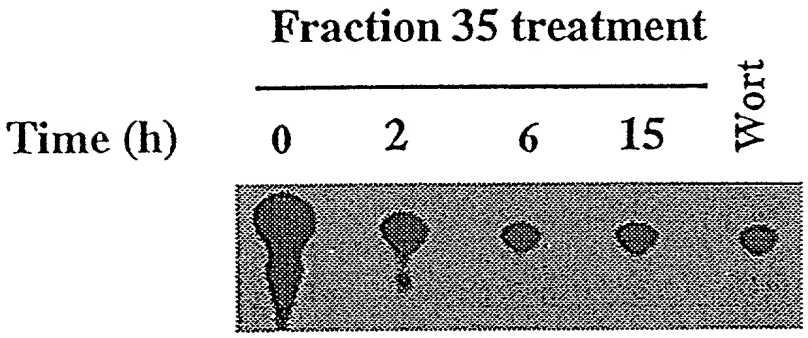


FIG 21

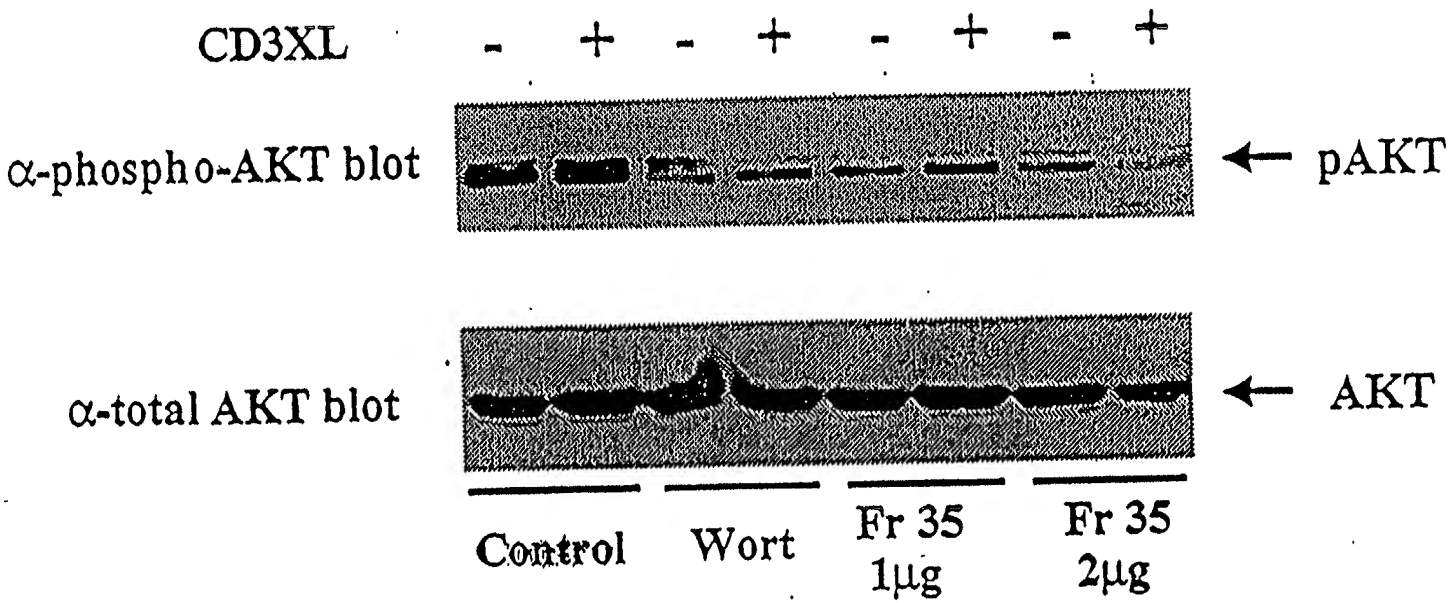

CD3XL
- + - + - + - +
α-phospho-AKT blot
pAKT
α-total AKT blot
AKT
Control
Wort
Fr 35 1µg
Fr 35 2µg

FIG 22

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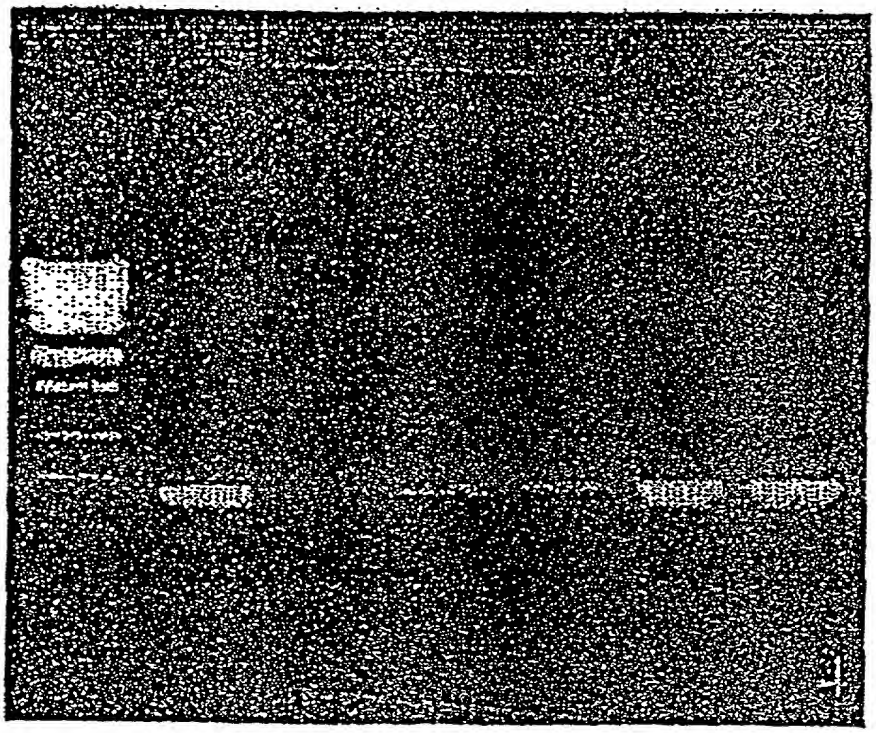


FIG 23

STRUCTURE OF ELLIPTOSIDES:

ELLIPTOSIDE E if R=OH

ELLIPTOSIDE A if R=H

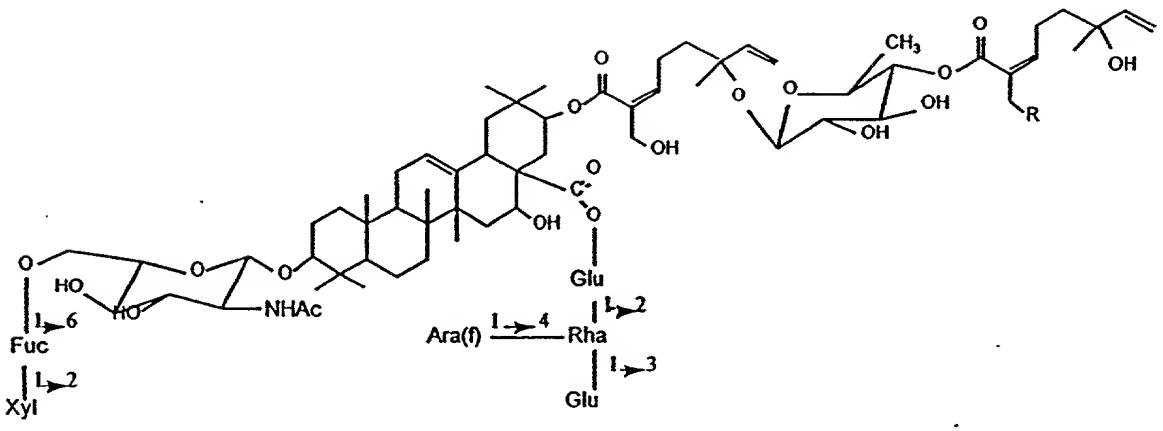
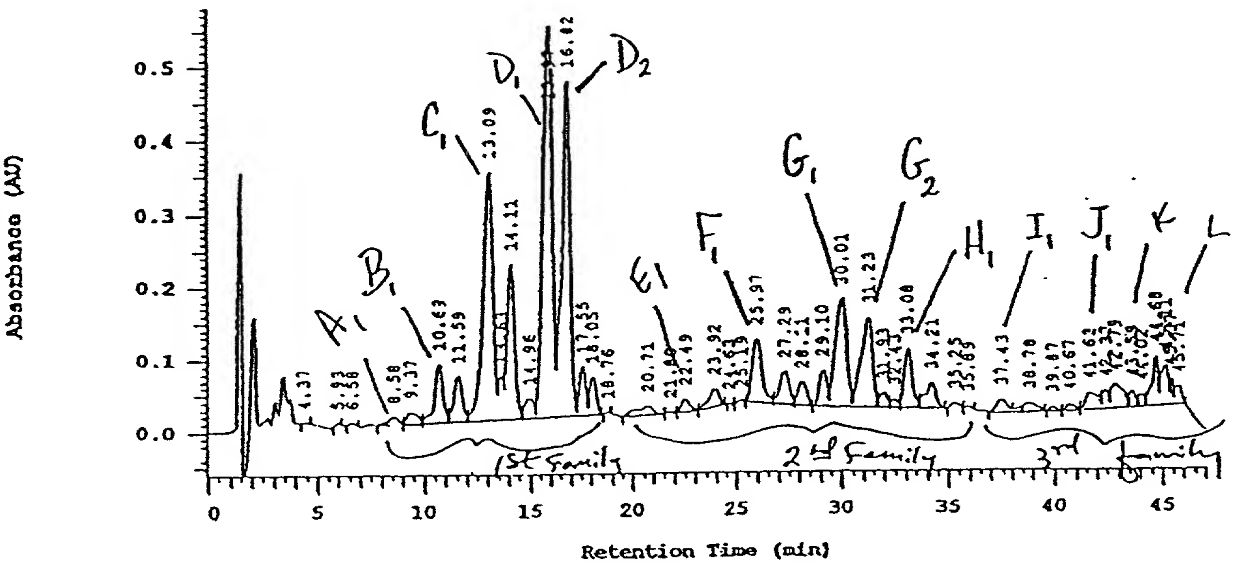
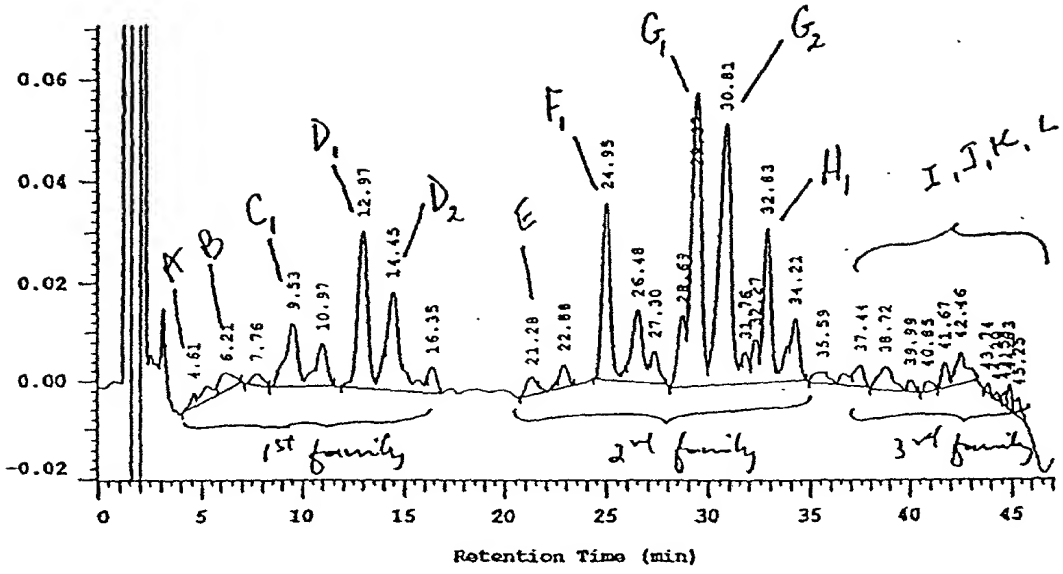


FIG 24



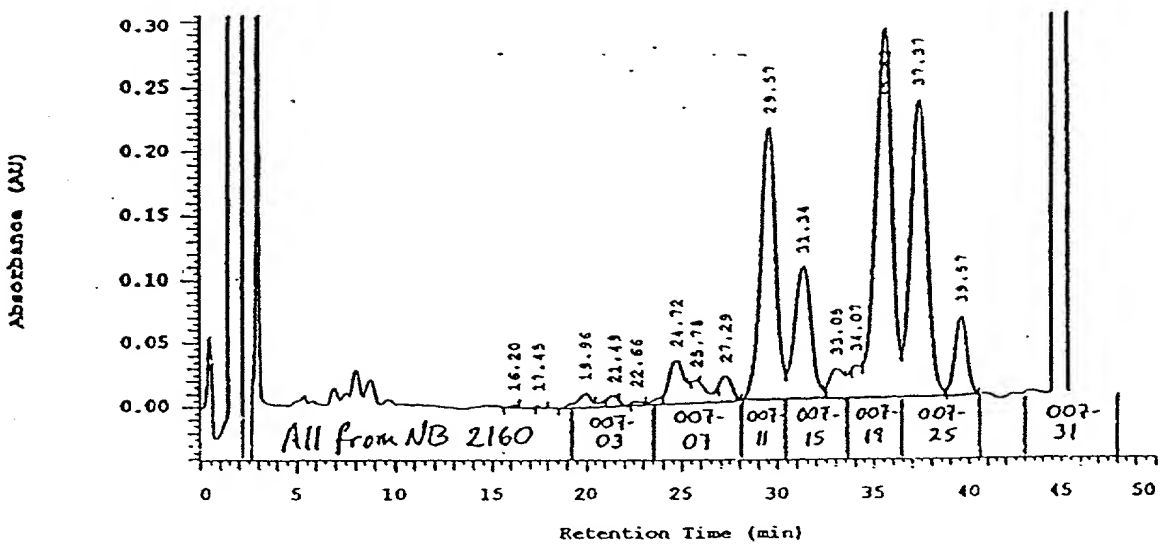
HPLC separation of the constituents in F094

FIG 25



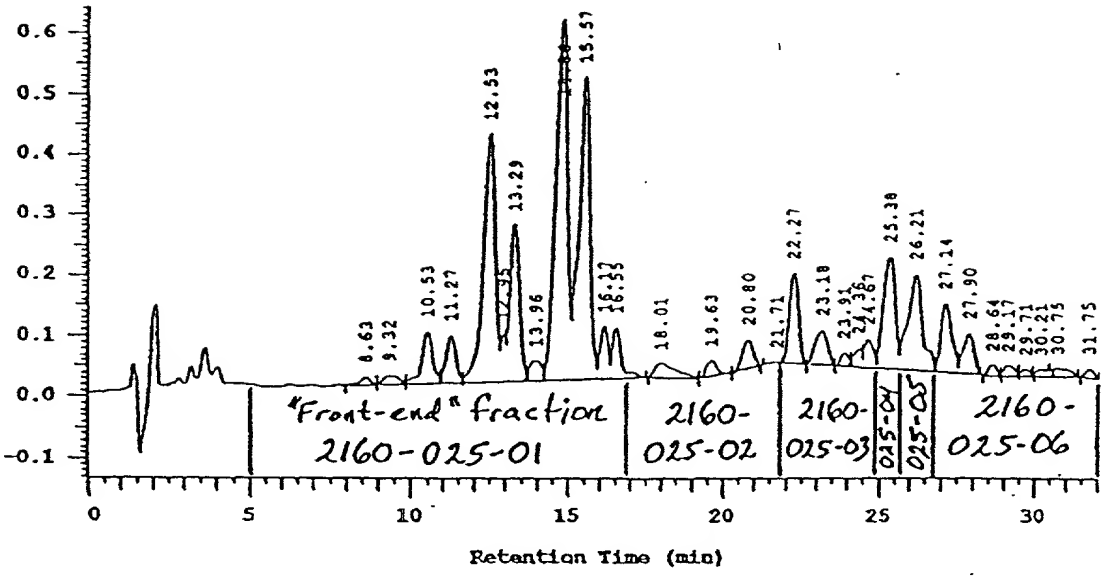
HPLC separation of the constituents in F035

FIG 26



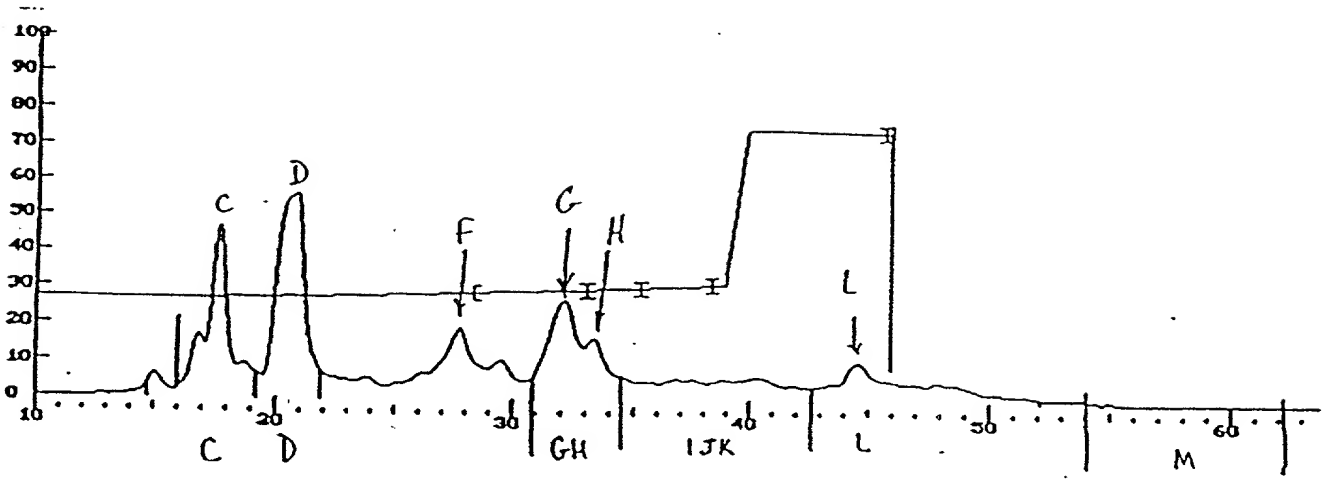
First Fractionation by Semi-Prep HPLC of F094

FIG 27



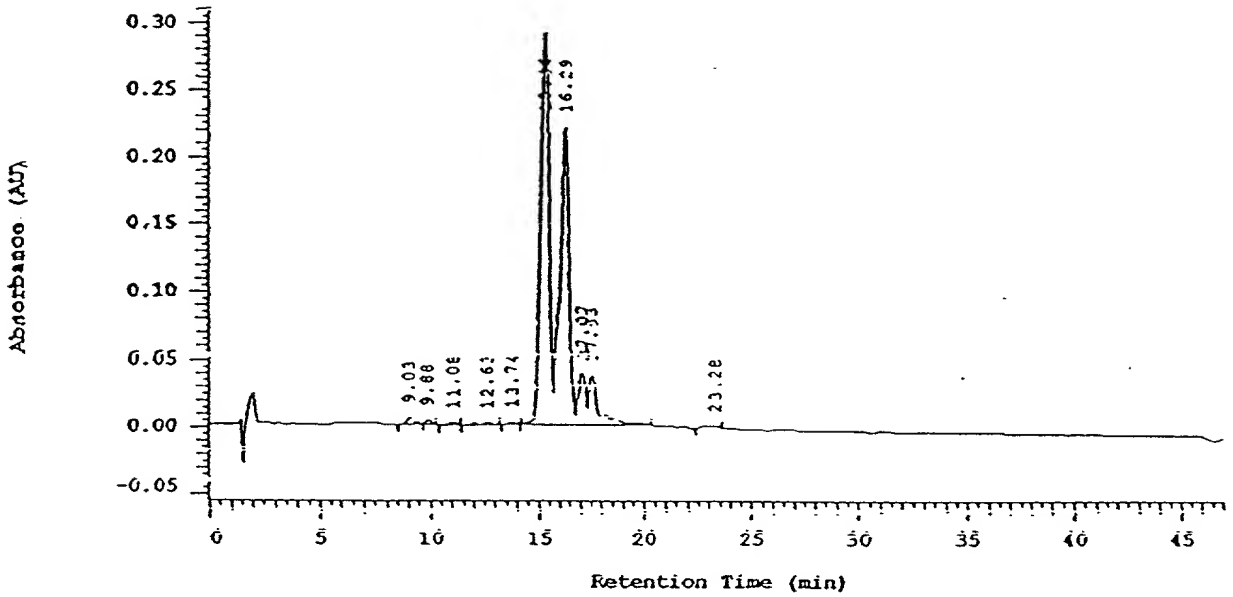
Second Fractionation by Semi-Prep HPLC of F094

FIG 28



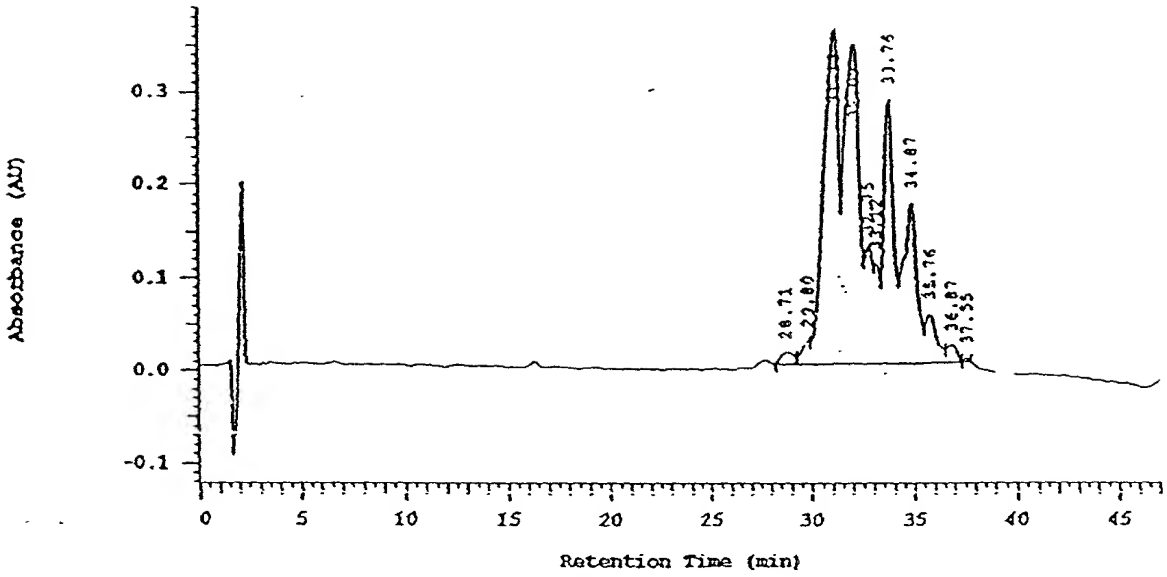
Prep Fractionation of F094

FIG 29



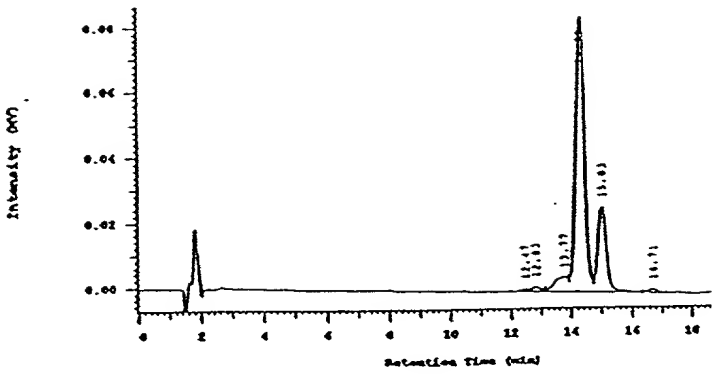
Analytical on Prep fraction D

FIG 30



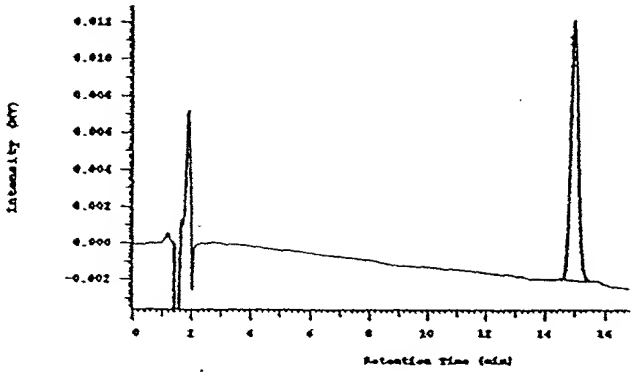
Analytical on Prep fraction G/H

FIG 31



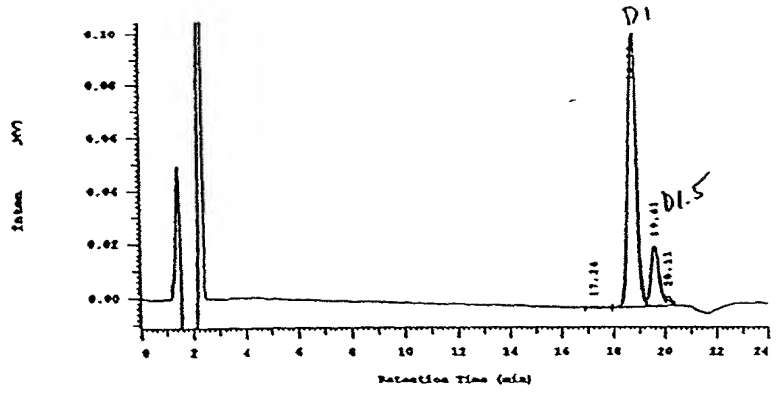
. G1 after second PFP column purification.

FIG 32



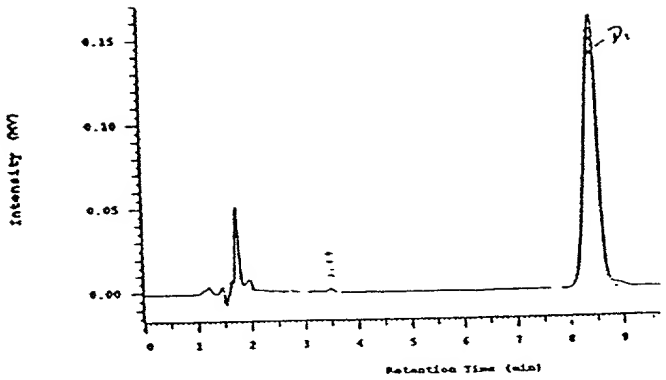
G1 after final C-18 purification

FIG 33



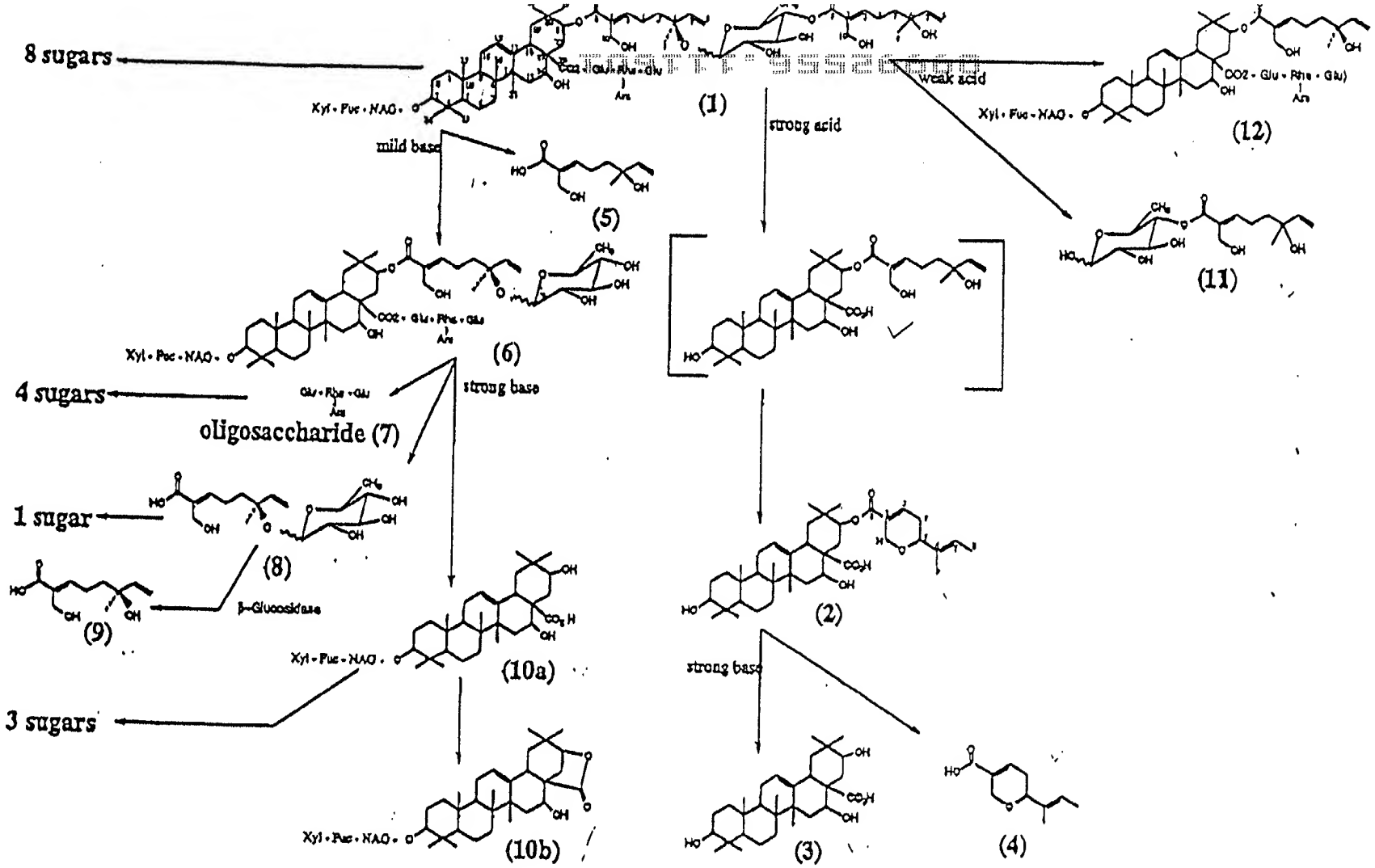
D1 after Waters C-18 column purification.

FIG 34



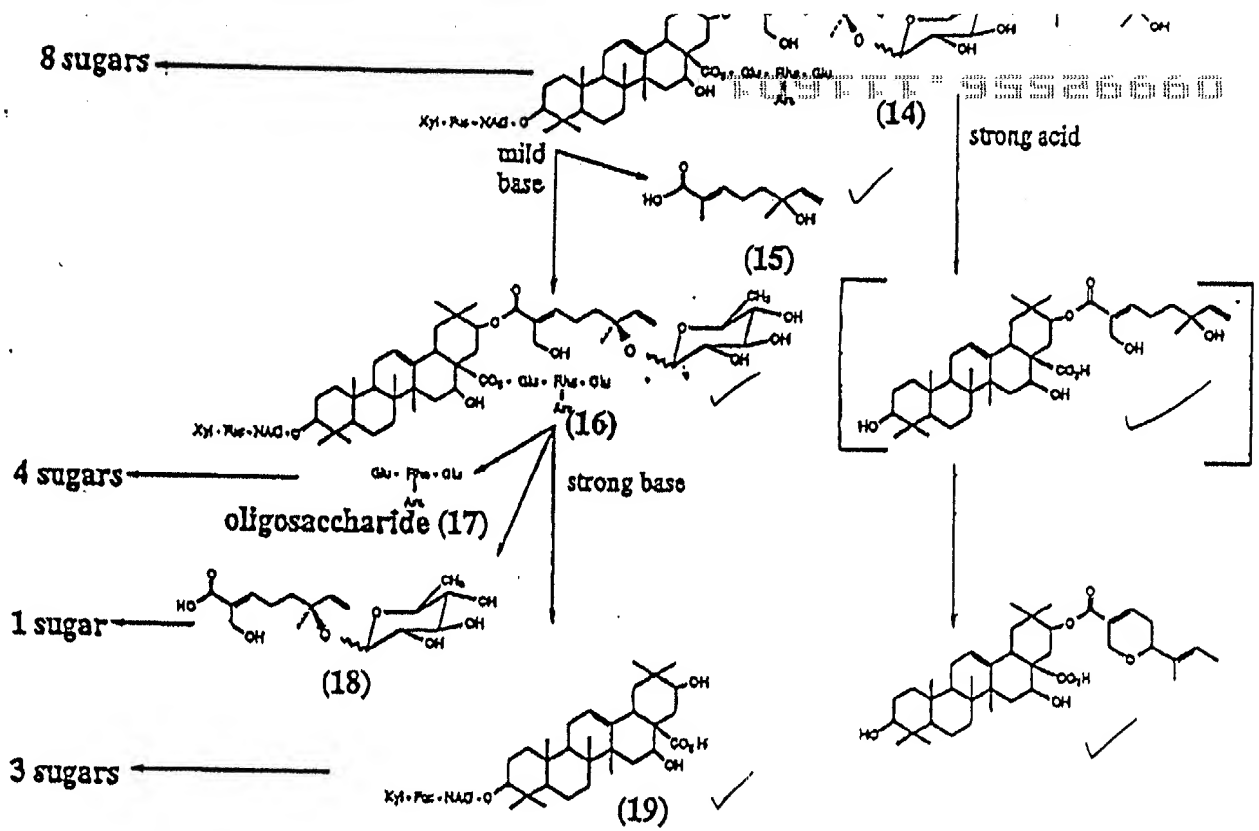
D1 after final C18-Aq purification

FIG 35



Compounds from the Degradation of D1

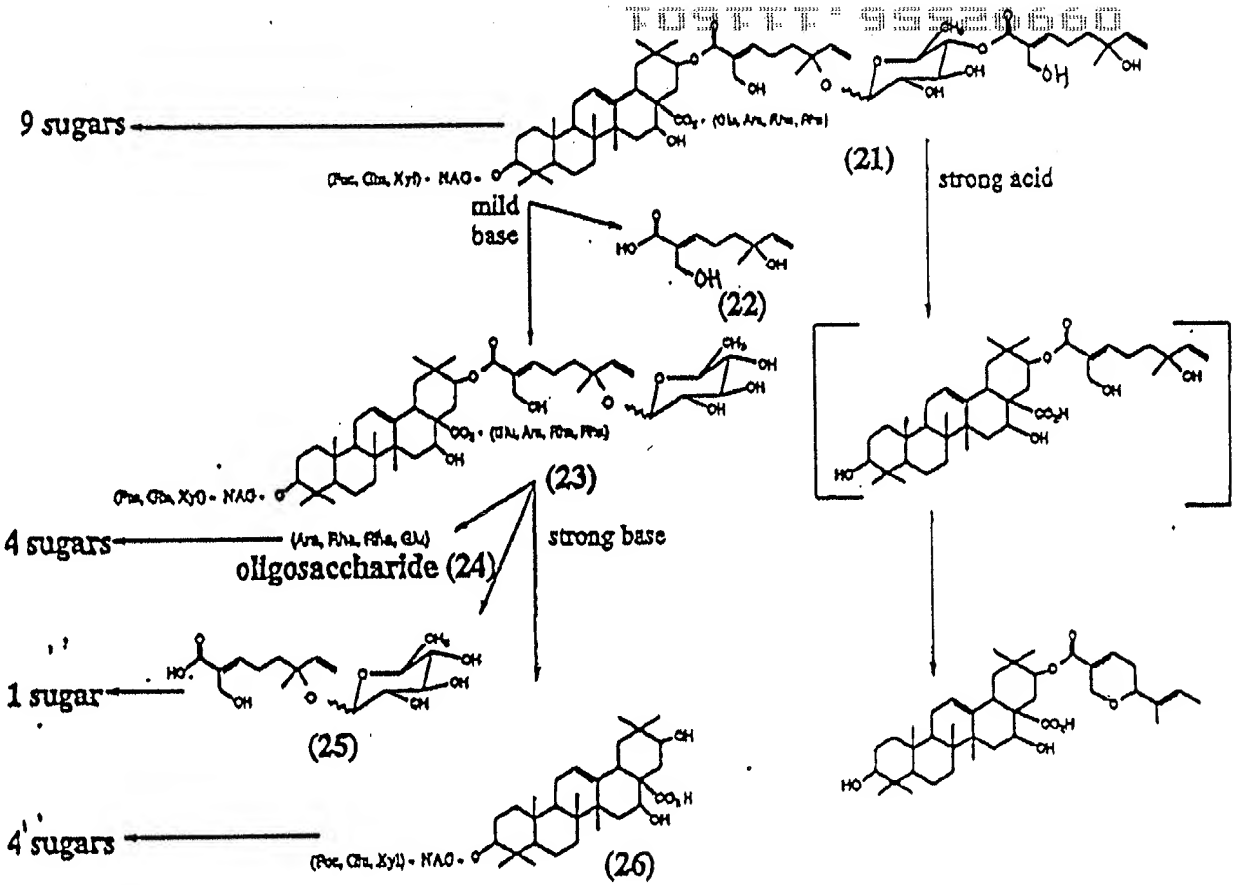
FIG 36



· Compounds from the Degradation of G1

FIG. 37

FOSTER-95230560



Compounds from the Degradation of B1

FIG. 38

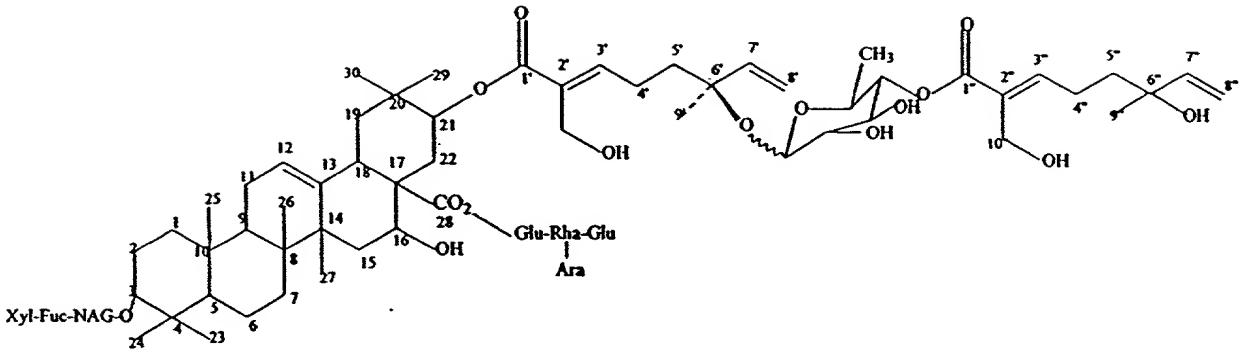


FIG. 39

Date Recue/Date Received 2020-05-15

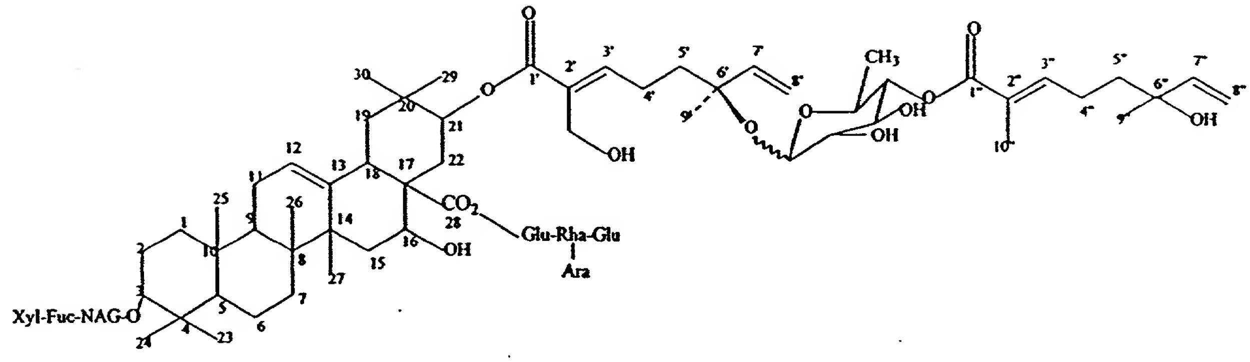


FIG. 40

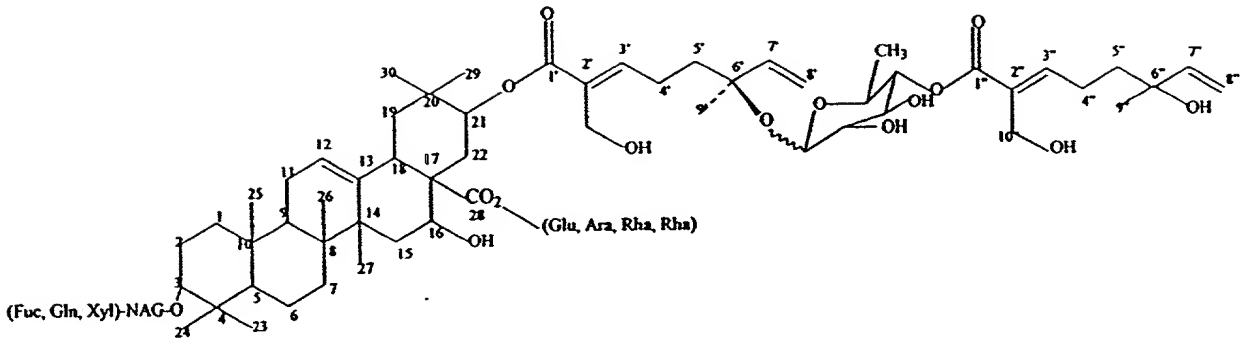


FIG. 41

A: 209800()
A 209800(#209800 v1 - CLFR 006 Figures -Drawn by Priya)

IC50 (µg / ml, * denotes values > 25 µg/ml)
0
2
4
6
8
10
12
14
16
18
20
JURKAT (T-cell leukemia)
OVCAR-3 (Ovarian)
C-2 (HEY VARIANT) (Ovarian)
Dov-13 (Ovarian)
SKOV-3 (Ovarian)
769-P (Renal)
786-O (Renal)
A-498 (Renal)
PANC-1 (Pancreatic)
PC-3 (Prostate)
DU145 (Prostate)
Lncap (Prostate)
MDA-MB-453 (Breast)
MDA-MB-435 (Breast)
MDA-MB-231 (Breast)
MDA-468 (Breast)
SKBR-3 (Breast)
BT-20 (Breast)
MCF-7 (Breast)
Hs27 (Human foreskin fibroblast)
L929 (Normal Mouse fibroblast)
MCF-10A (Normal Breast Epithelium)
MCF-10F(Normal Breast Epithelium)
*
*
*
*
Cell Lines

FIG. 42

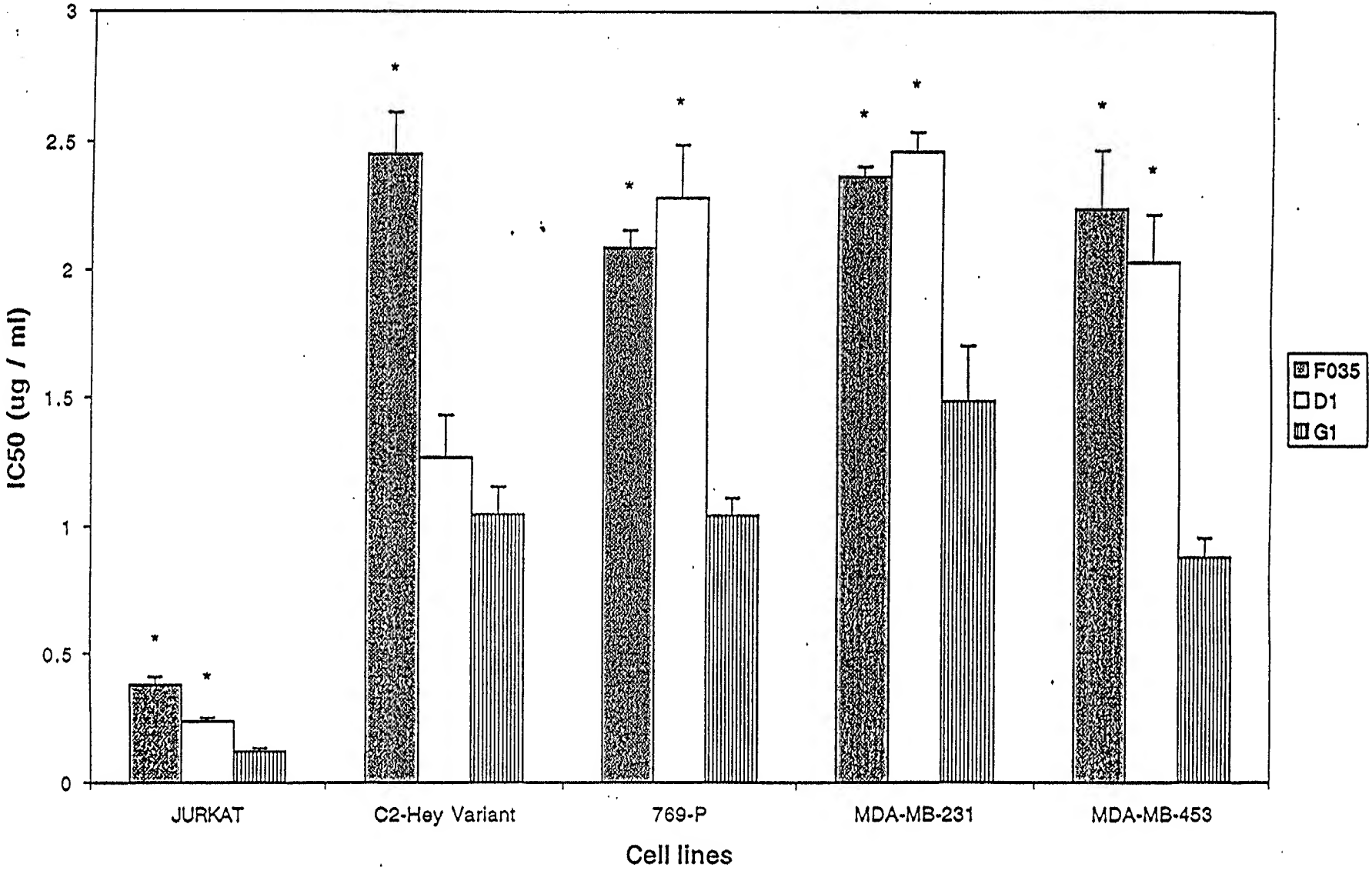

3
2.5
2
1.5
1
0.5
0
IC50 (ug / ml)
JURKAT
C2-Hey Variant
769-P
MDA-MB-231
MDA-MB-453
Cell lines
F035
D1
G1

FIG 43

FOSTER 95526660

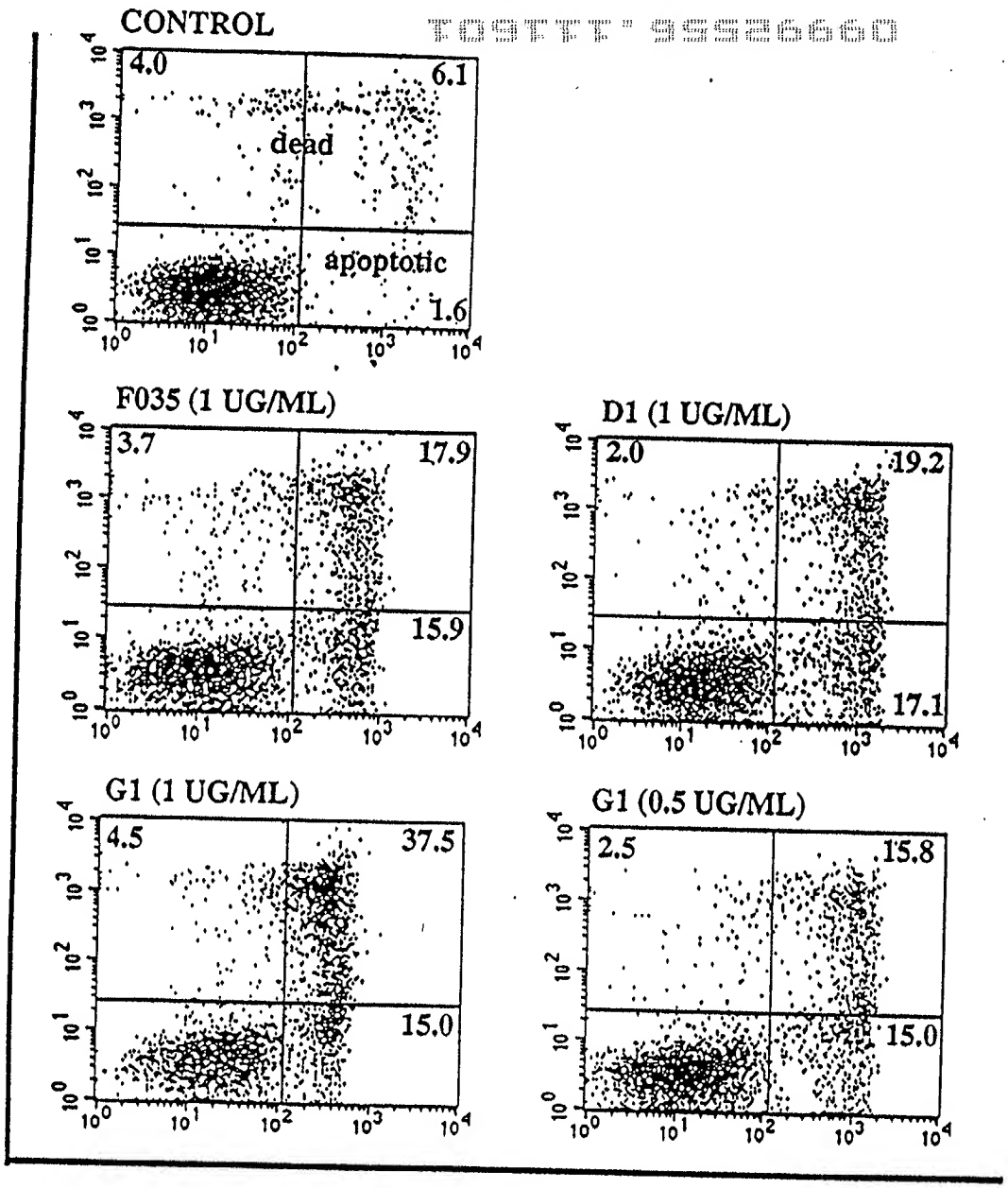
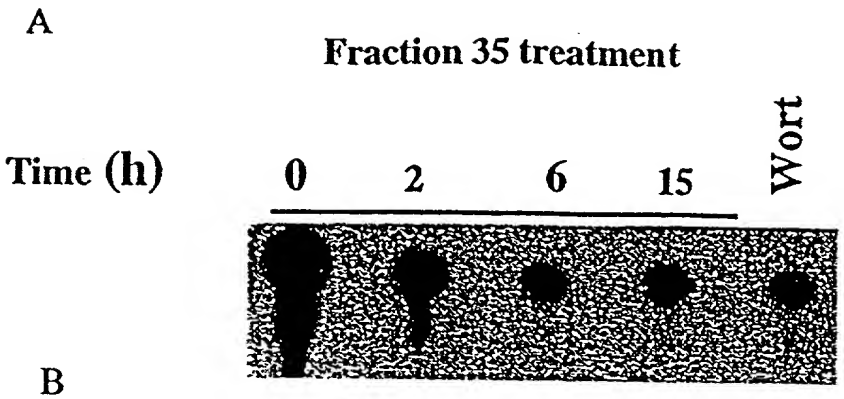


FIG 44

A


Fraction 35 treatment
Time (h)
0
2
6
15
Wort

B

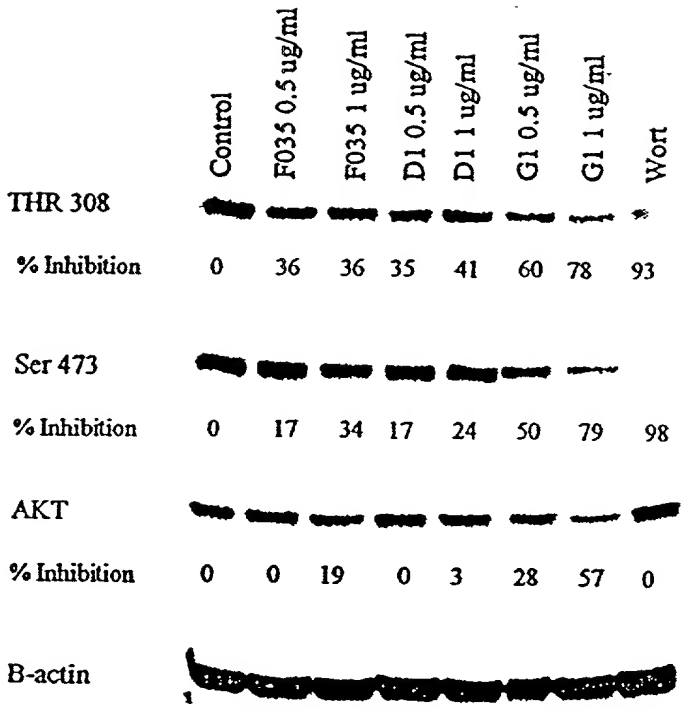
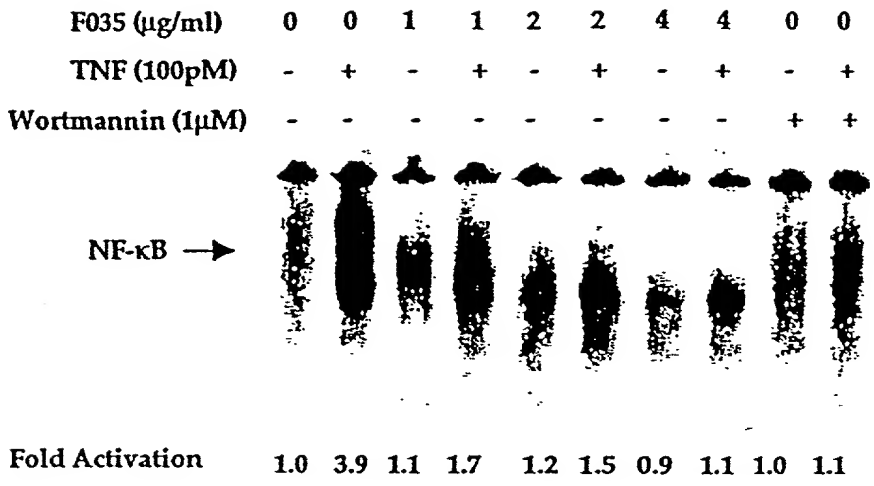
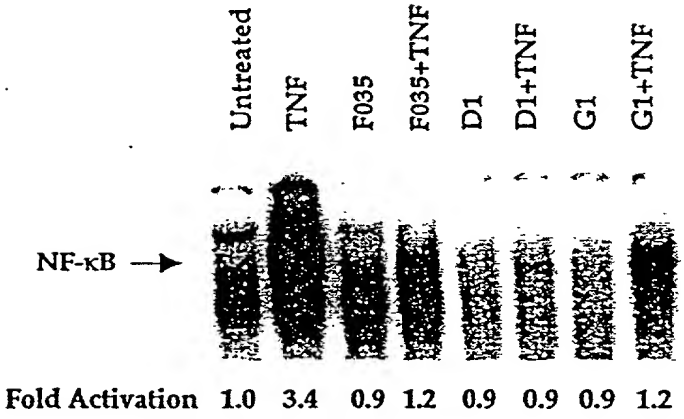

Control
F035 0.5 ug/ml
F035 1 ug/ml
D1 0.5 ug/ml
D1 1 ug/ml
G1 0.5 ug/ml
G1 1 ug/ml
Wort
THR 308
% Inhibition 0 36 36 35 41 60 78 93
Ser 473
% Inhibition 0 17 34 17 24 50 79 98
AKT
% Inhibition 0 0 19 0 3 28 57 0
B-actin

FIG 45

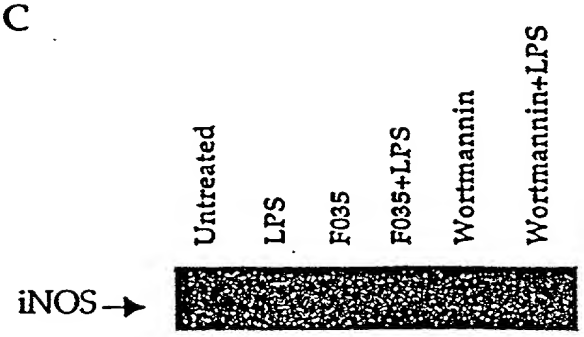
A


F035 (μg/ml) 0 0 1 1 2 2 4 4 0 0
TNF (100pM) - + - + - + - + - +
Wortmannin (1μM) - - - - - - - - + +
NF-κB
Fold Activation 1.0 3.9 1.1 1.7 1.2 1.5 0.9 1.1 1.0 1.1

B


Untreated
TNF
F035
F035+TNF
D1
D1+TNF
G1
G1+TNF
NF-κB
Fold Activation 1.0 3.4 0.9 1.2 0.9 0.9 0.9 1.2

C


Untreated
LPS
F035
F035+LPS
Wortmannin
Wortmannin+LPS
iNOS

D

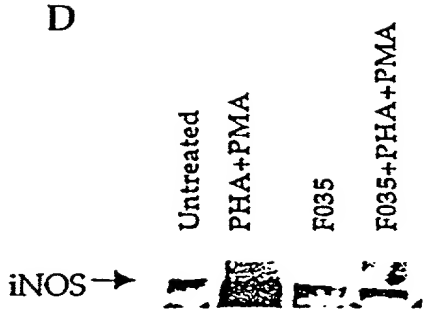

Untreated
PHA+PMA
F035
F035+PHA+PMA
iNOS

FIG 46

Effect of F035 & D1 on cleavage of PARP in Jurkat cells.

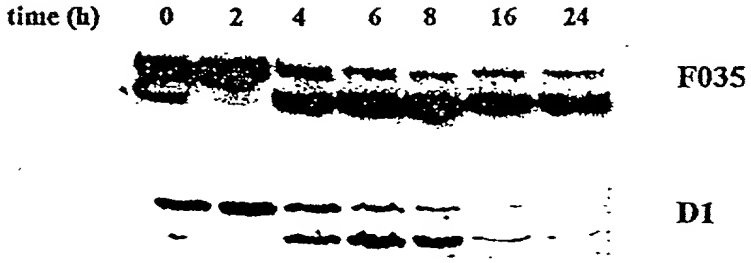


FIG 47

Effect of z-vad fmk on F035 induced PARP cleavage in Jurkat cells

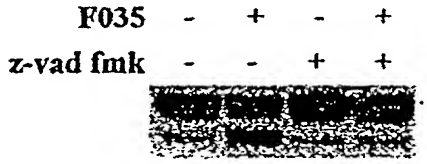


FIG 48

Effect of F035, F094, D1 & G1 on caspase activity in Jurkat cells.

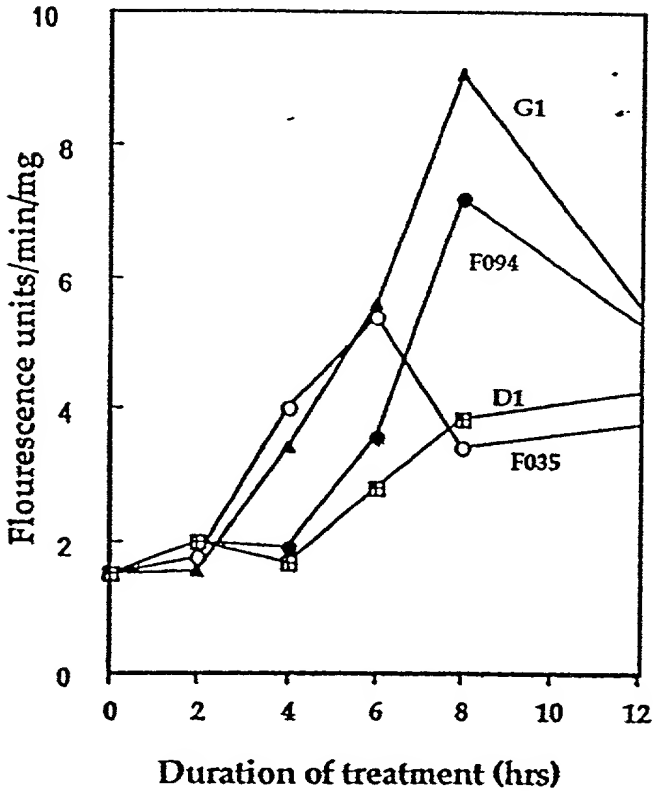


FIG 49

Effect of F035 on cytochrome c release from Jurkat mitochondria

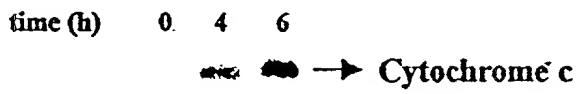
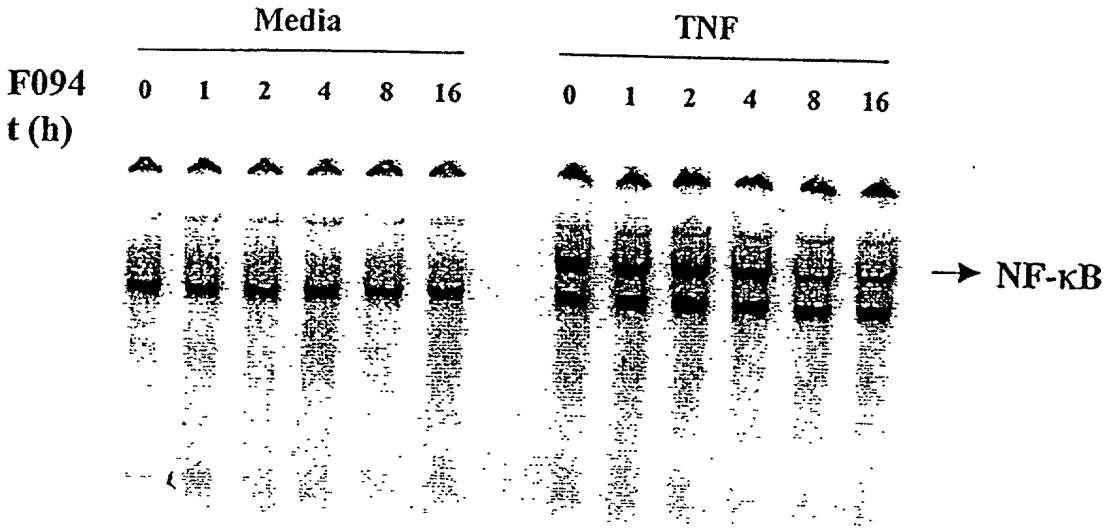


FIG 50

A


Media
TNF
F094
t (h)
0 1 2 4 8 16
0 1 2 4 8 16
NF-κB

B

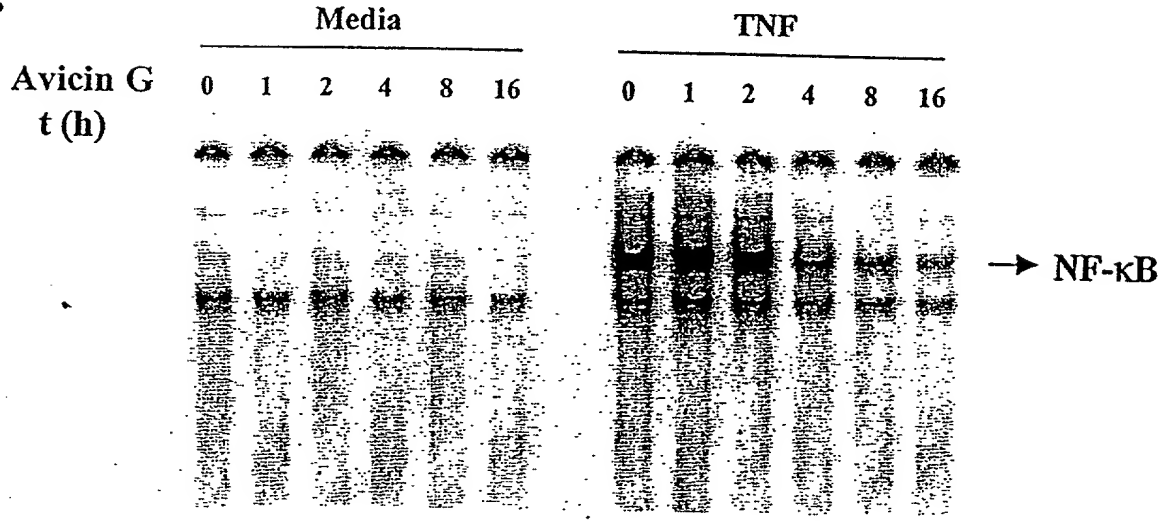
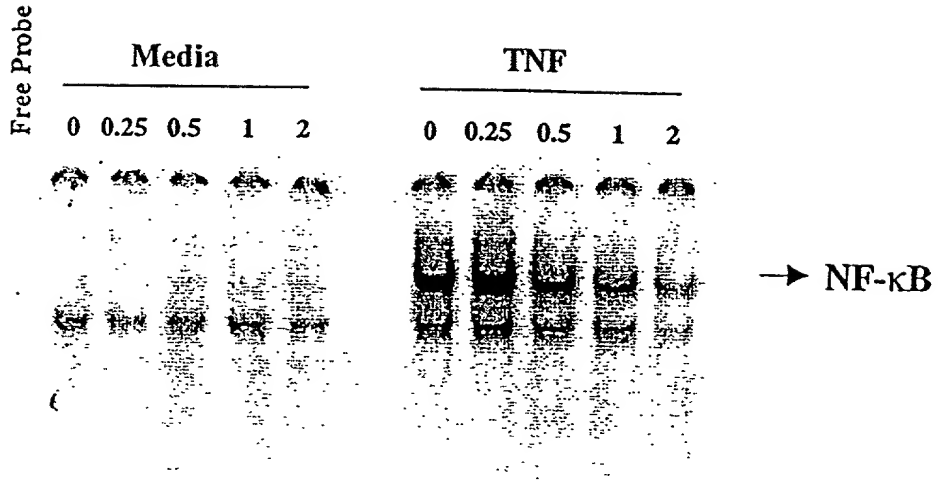

Media
TNF
Avicin G
t (h)
0 1 2 4 8 16
0 1 2 4 8 16
NF-κB

FIG. 51 A-B

A



B

Rabbit IgG	-	-	-	-	-	+
anti-p65	-	-	-	-	+	-
Cold wt	-	-	-	+	-	-
Cold mut	-	-	+	-	-	-
TNF	-	+	+	+	+	+

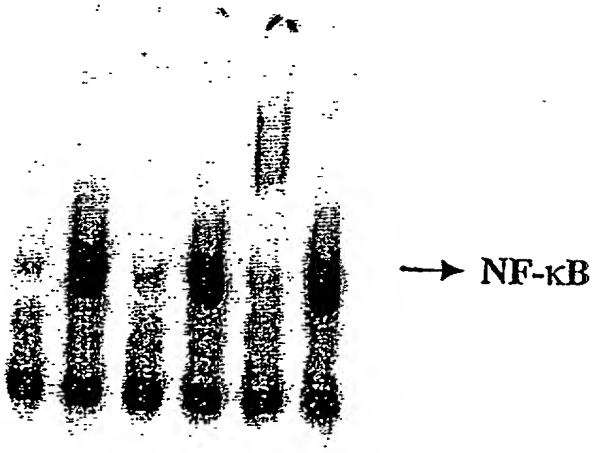
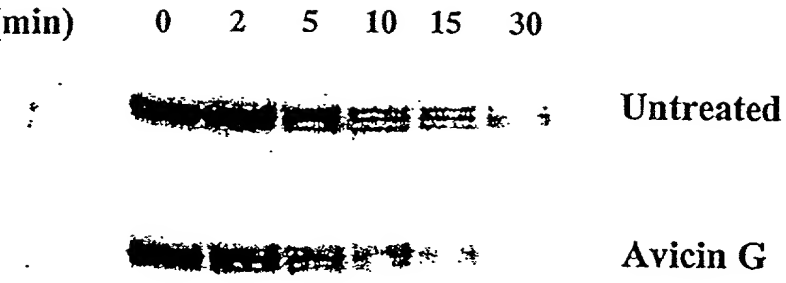


FIG. 52 A-B

A IκB



B p65

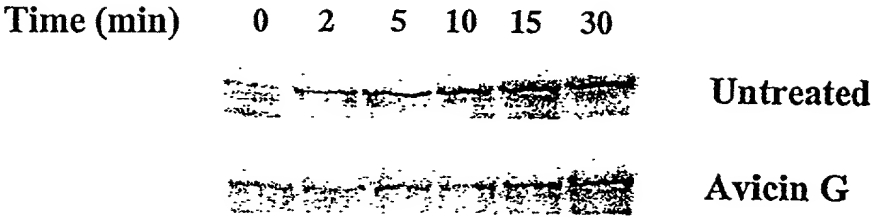
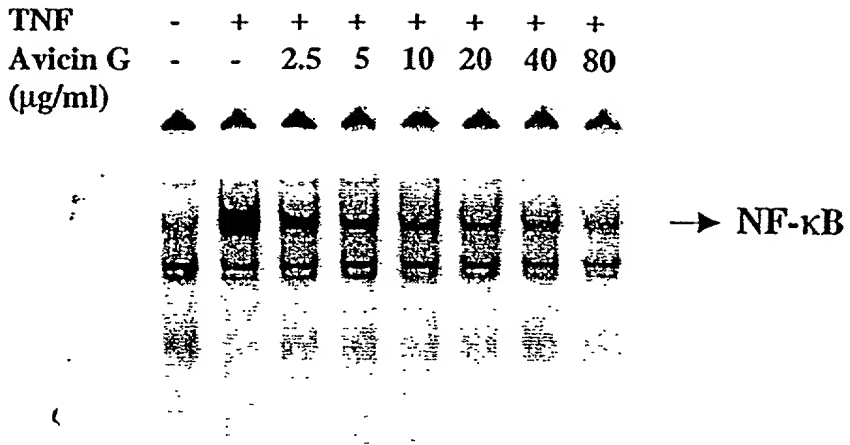
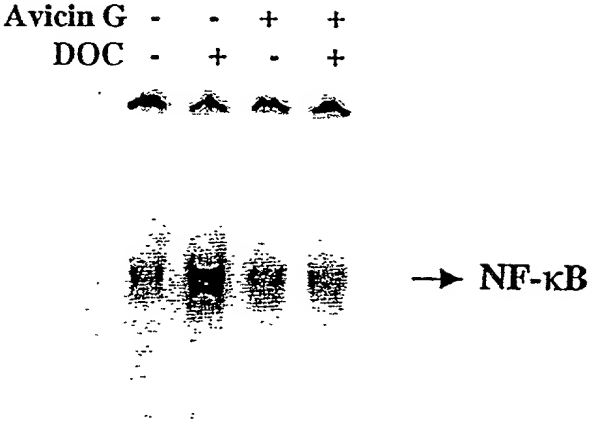


FIG. 53 A-B

A



B



C

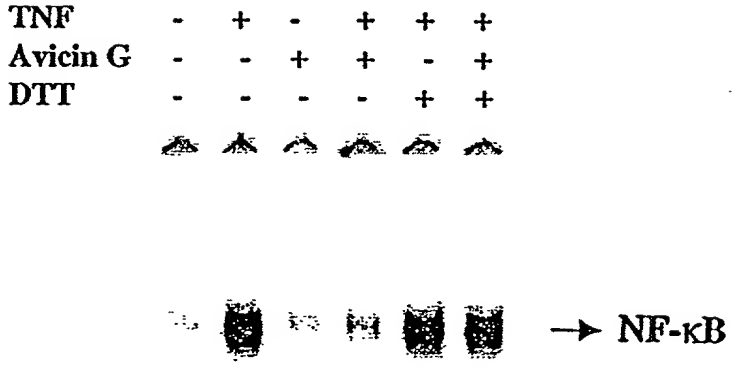
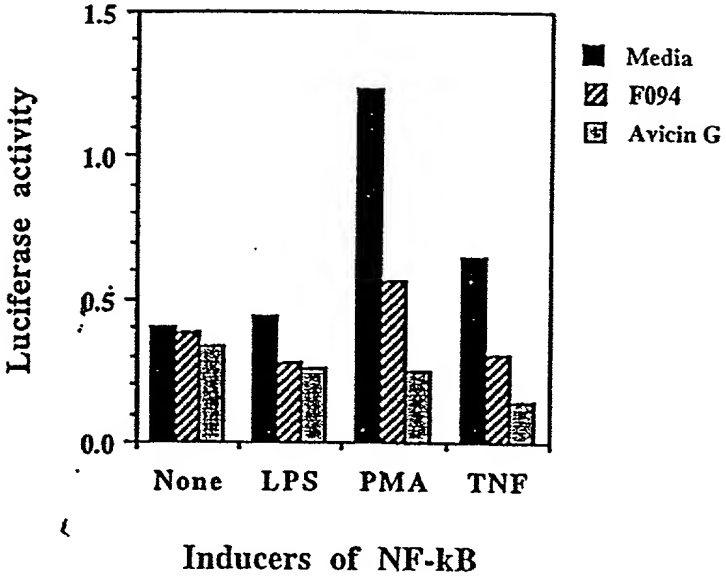


FIG. 54 A, B, C

A


1.5
1.0
0.5
0.0
Luciferase activity
Media
F094
Avicin G
None
LPS
PMA
TNF
Inducers of NF-kB

B

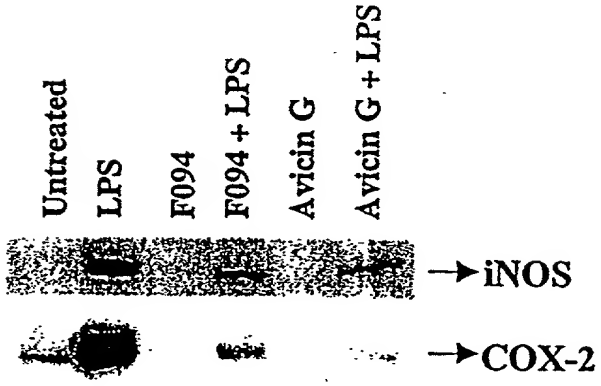
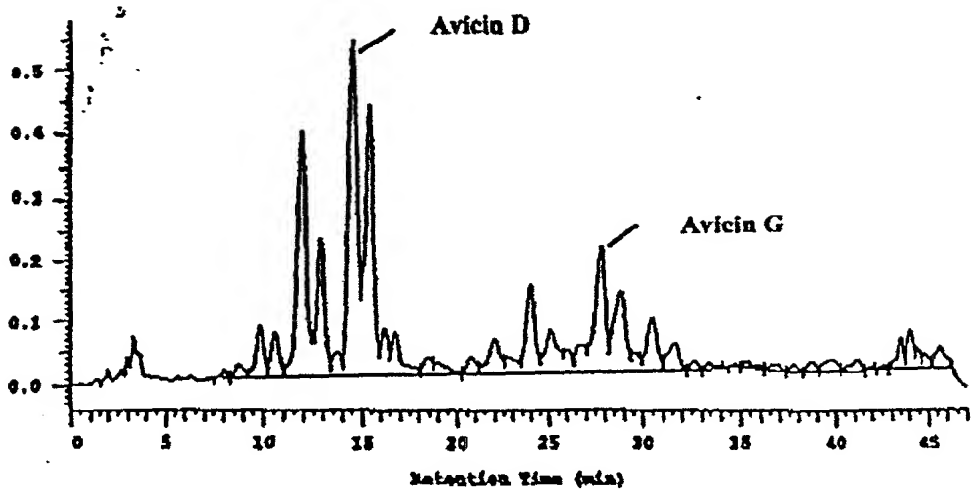

Untreated
LPS
F094
F094 + LPS
Avicin G
Avicin G + LPS
iNOS
COX-2

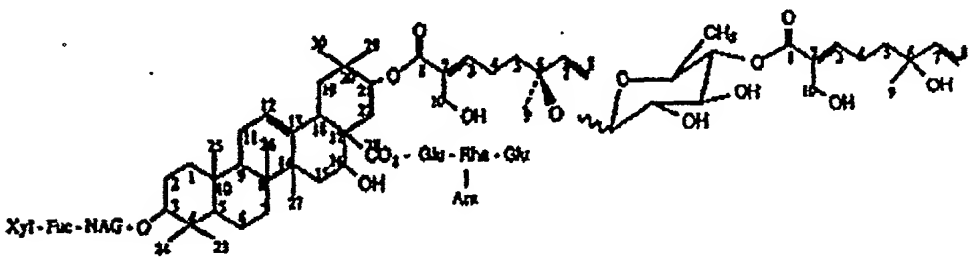
FIG. 55 A, B



HPLC Separation of the Avicins in F094

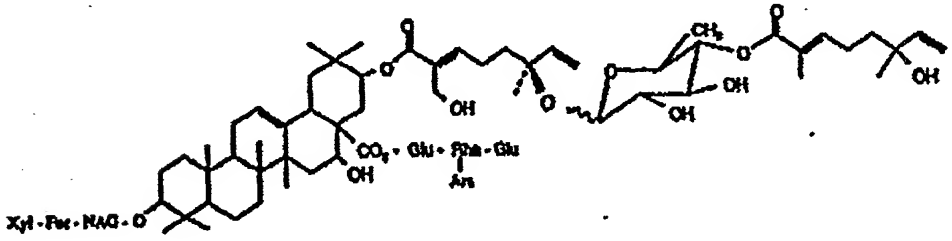
IC_{50} Values

0.331-0.407 µg/ml



Avicin D

0.320-0.326 µg/ml



Avicin G

0.160-0.181 µg/ml

FIG. 56

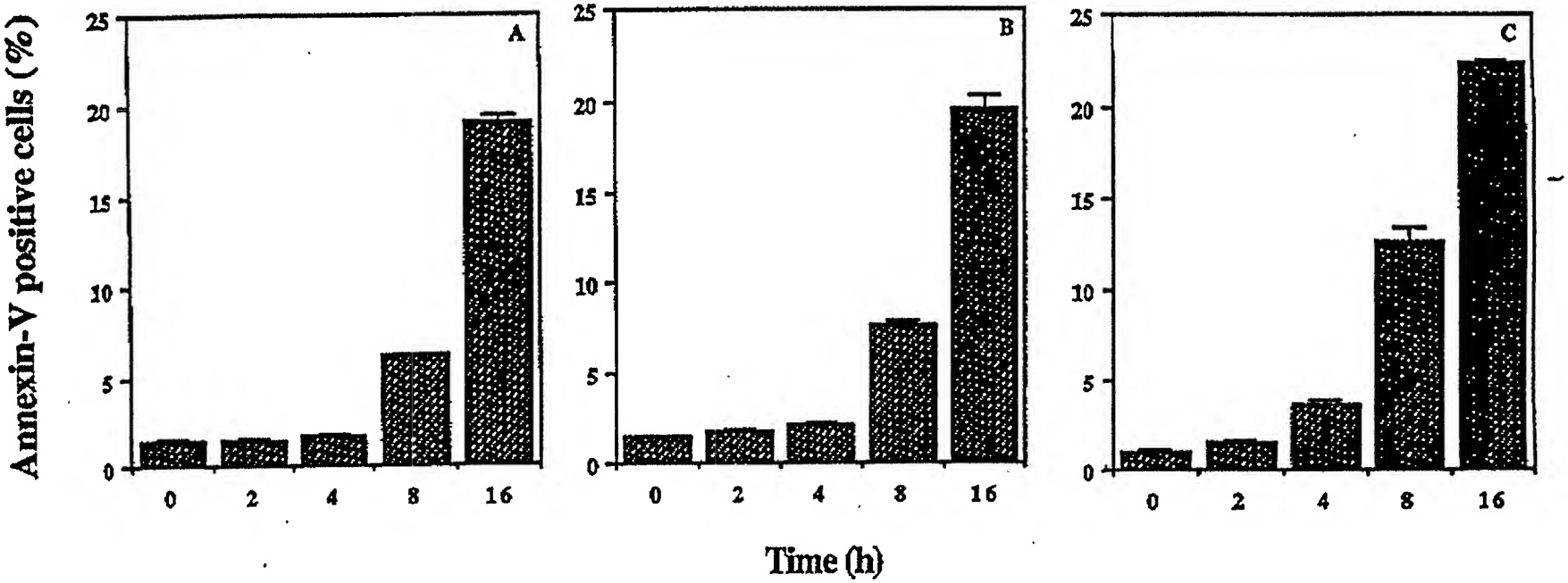


FIG. 57

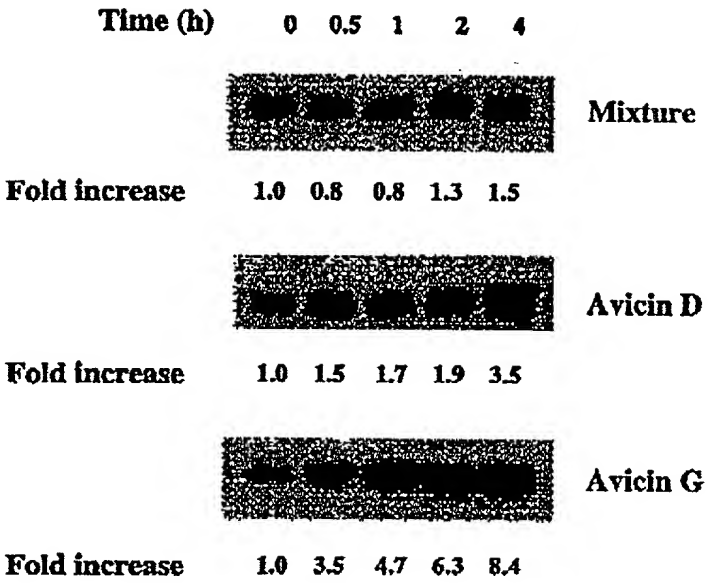

Time (h) 0 0.5 1 2 4
Mixture
Fold increase 1.0 0.8 0.8 1.3 1.5
Avicin D
Fold increase 1.0 1.5 1.7 1.9 3.5
Avicin G
Fold increase 1.0 3.5 4.7 6.3 8.4

FIG. 58

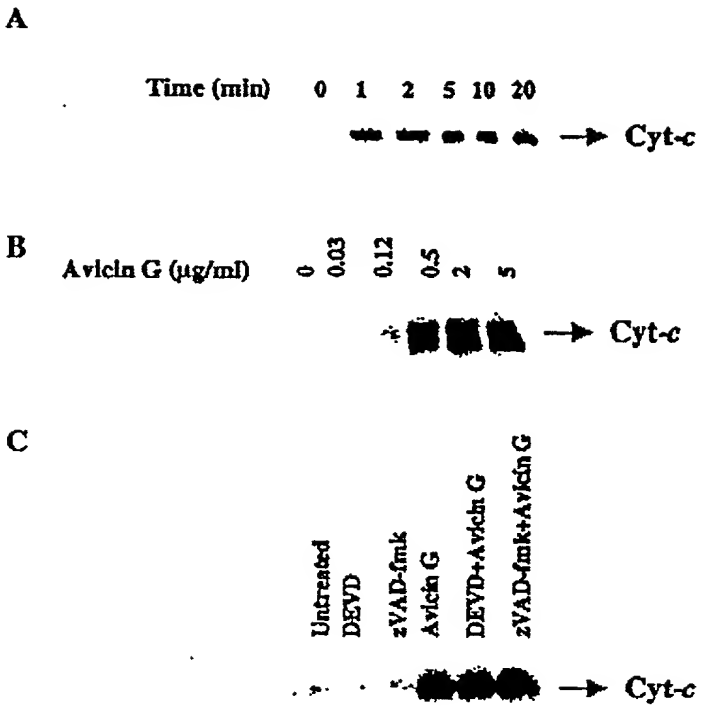

A
Time (min) 0 1 2 5 10 20
Cyt-c
B
Avicin G (μg/ml) 0 0.03 0.12 0.5 2 5
Cyt-c
C
Untreated
DEVD
zVAD-fmk
Avicin G
DEVD+Avicin G
zVAD-fmk+Avicin G
Cyt-c

FIG. 59

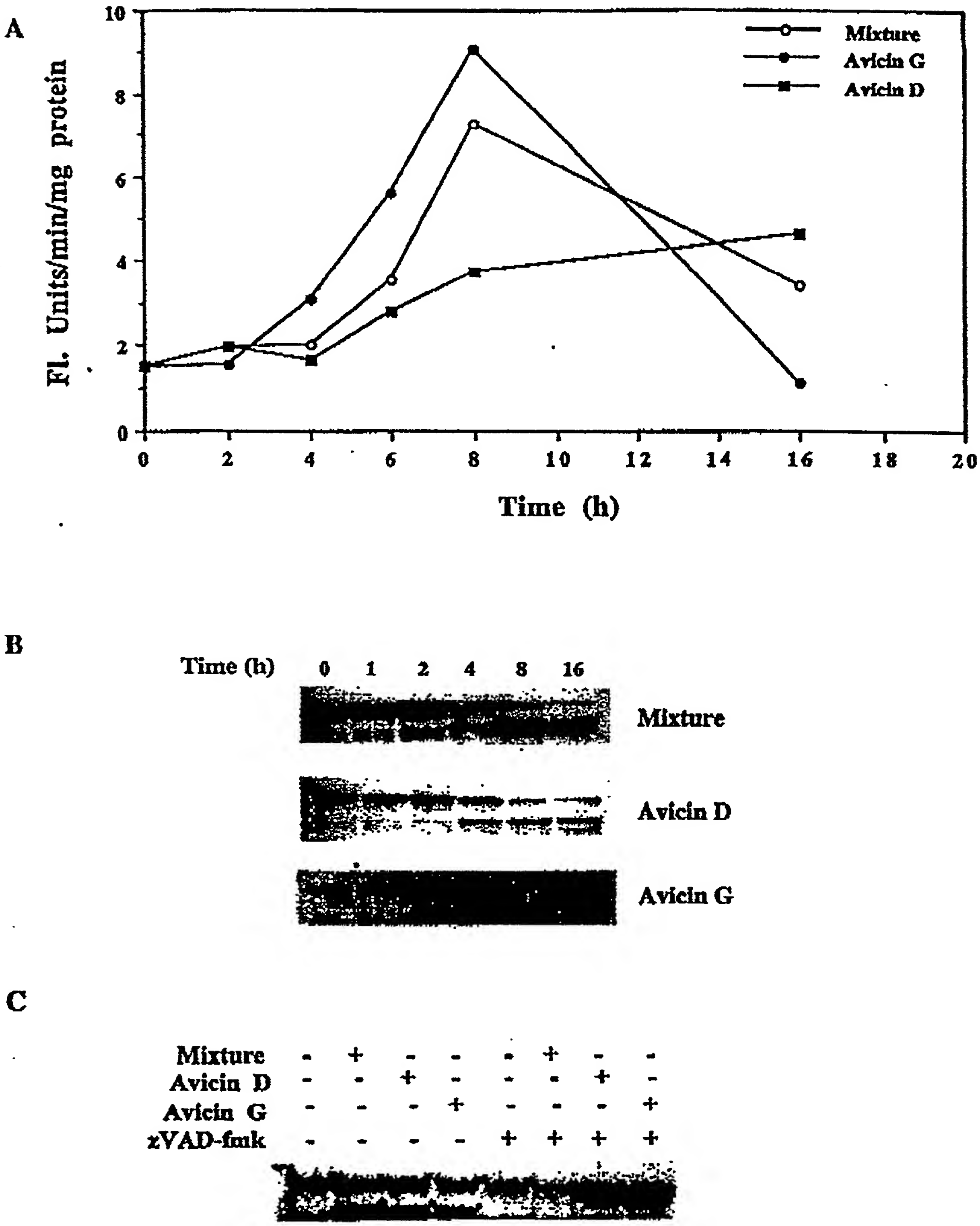

A
Fl. Units/min/mg protein
Time (h)
Mixture
Avicin G
Avicin D
0
2
4
6
8
10
12
14
16
18
20
B
Time (h)
0 1 2 4 8 16
Mixture
Avicin D
Avicin G
C
Mixture
Avicin D
Avicin G
zVAD-fmk

FIG. 60

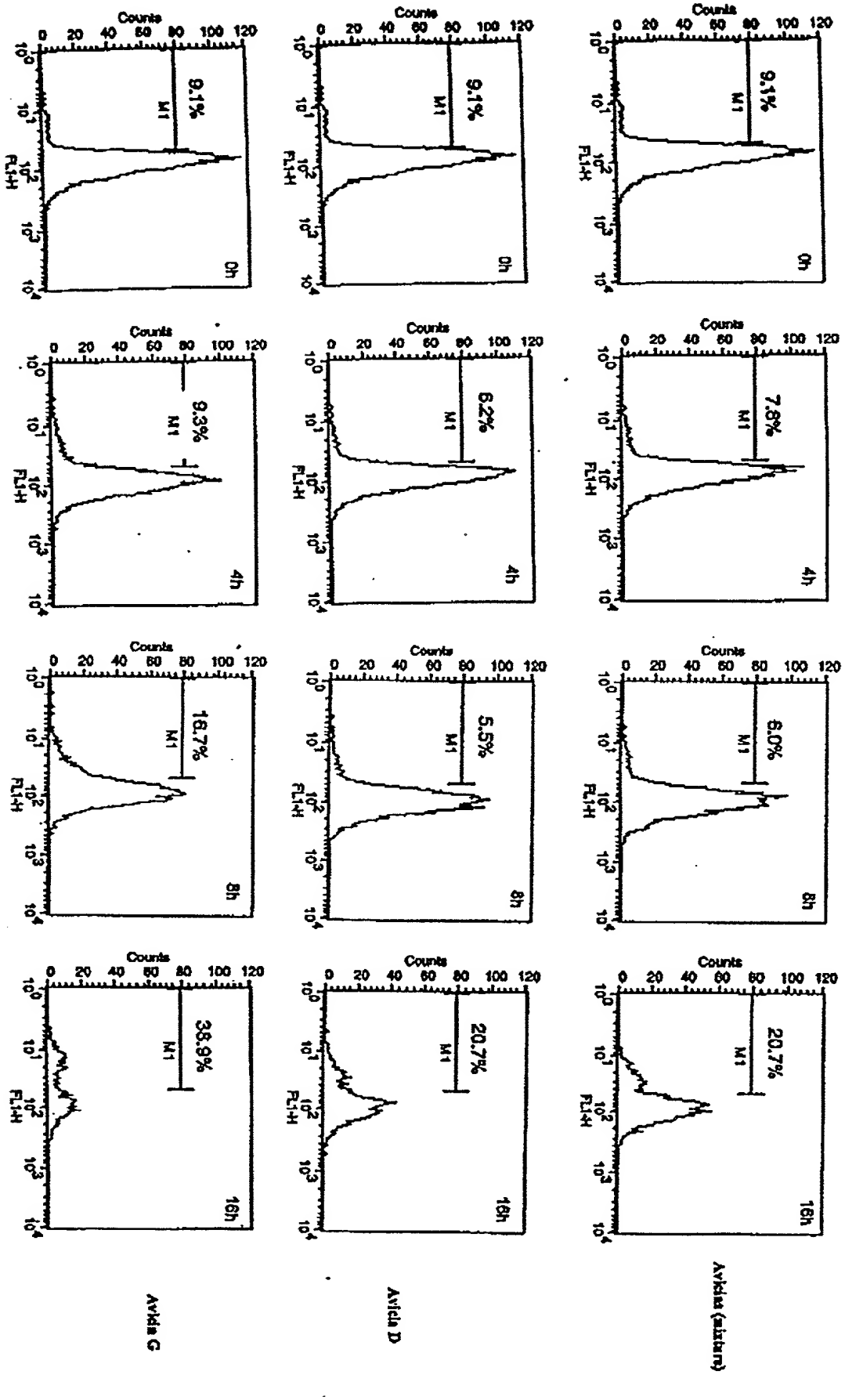

Counts
FL1-H
M1
9.1%
0h
9.1%
0h
9.1%
0h
9.3%
4h
6.2%
4h
7.8%
4h
16.7%
8h
5.5%
8h
6.0%
8h
38.9%
16h
20.7%
16h
20.7%
16h
Avidis G
Avidis D
Avidis (mixture)

FIG. 61

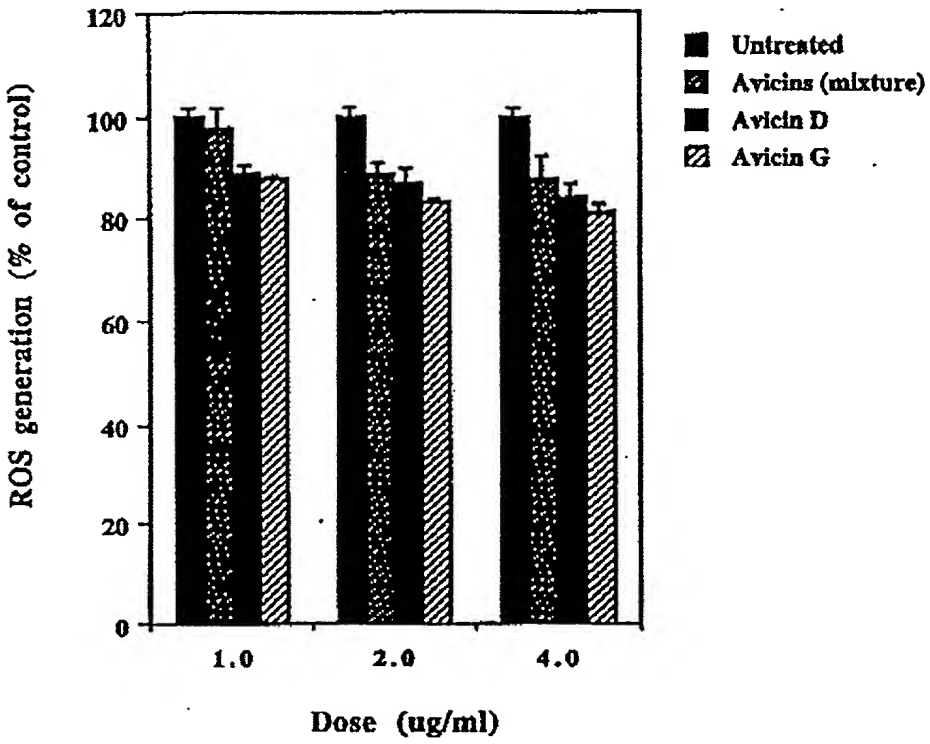

120
100
80
60
40
20
0
ROS generation (% of control)
1.0
2.0
4.0
Dose (ug/ml)
Untreated
Avicins (mixture)
Avicin D
Avicin G

FIG. 62